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AN ASIATIC SPECIES OF GYMNOSPORANGIUM ESTABLISHED IN OREGON [1]

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INTRODUCTION

Early in June, 1914, specimens of a species of Roestelia on Japanese pear leaves were sent to the writer from the office of the Secretary of the Oregon State Board of Horticulture. These had been collected in the yard of a Japanese family at Orient, in the vicinity of Portland, Oreg. The writer visited the locality on June 11, 1914, and found two Japanese pear trees (*Pyrus sinensis*) the foliage of which was seriously affected with the fungus (Pl. LXXVIII, fig. 1). Since all species of Roestelia, so far as known, are the æcial stages of species of Gymnosporangium, and none are known to be perennial, it was at once recognized that the source of infection must be in the immediate vicinity. A search was made for a possible telial stage, but no positive evidence of the occurrence of such was obtained at that time, on account of the lateness of the season, though several varieties of Juniperus, as well as other members of the Juniperaceae, were found growing in the same yard, all of which were stated by the owners to have been directly imported from Japan several years before. Inquiry revealed that the rust had been present in small amount the previous season.

Careful examination showed that the rust should properly be referred to *Roestelia koreaensis* P. Henn., which was originally described from material collected in Korea (Chosen), but has since been reported as occurring commonly in Japan. An examination of the literature showed that considerable confusion has existed regarding the identity and relationship of certain of the Asiatic species of Gymnosporangium. Two species

[1] This paper is based on studies which were conducted in the laboratory of the Department of Botany and Plant Pathology of the Oregon Agricultural College Experiment Station. It is essentially as read at the summer meeting of the American Phytopathological Society, at Berkeley, Cal., on August 5, 1915, with certain additional information obtained from the examination of material in the herbarium of Dr. J. C. Arthur, to whom grateful acknowledgment is due for this privilege as well as for helpful suggestions. See *abstracts in* Phytopathology, v. 5, no. 5, p. 293, 1915, and Science, n. s., v. 42, no. 1086, p. 582, 1915.

have been especially confused, and on account of their interest in North America they will be discussed together in this paper. In order to make the situation clear, a review of the literature of these rusts with reference to their occurrence in Japan as well as in the United States will be given.

INVESTIGATIONS IN JAPAN

From 1897 to 1899 Shirai (7)[1] conducted infection experiments in which he claimed to show that *Roestelia koreaensis* was genetically connected with *Gymnosporangium japonicum* Sydow. He succeeded, in several different experiments, in obtaining the development of typical æcia of *R. koreaensis* on the leaves of *Pyrus sinensis* by exposing them to infection from germinating telia on *Juniperus chinensis*. Shirai stated, however, that in Japan the telia of *G. japonicum* occur not only on the trunks and branches, as the original diagnosis of Sydow states, but also on the leaves of the juniper, and he described and figured both stages (7, pl. 1, fig. 19 and 22).

Ito (4) recently called attention to the fact that Japanese mycologists have for some time considered that the forms which occur on the stem and leaves of *Juniperus chinensis* are not the same species. He also recorded the results of infection experiments in which the teliospores of the stem form were sown on *Pyrus sinensis*, *Amelanchier asiatica*, and *Pourthiaea villosa*, with infection only on the last. The resulting æcia proved to be typical of *Roestelia photiniae* P. Henn. Referring to the leaf form, Ito further stated that he considered it to be *G. Haraeanum* Syd. and that *G. asiaticum* Miyabe is synonymous. Miyabe and Yamada (6) have recently shown by infection experiments that *G. asiaticum*, which occurs on the leaves of *J. chinensis*, has for its æcial stage a species of Roestelia on *Pyrus sinensis*, *Cydonia vulgaris*, and *Cydonia japonica*. Hara (3) has also recently shown by infection experiments that *G. Haraeanum* has for its æcial stage *R. koreaensis* on *Pyrus sinensis*.

From the above it would appear that Shirai had both forms, *Gymnosporangium japonicum* and *G. Haraeanum*, mixed in the material which he used for inoculation and that his successful results on the pear were due to infection by the sporidia of the leaf form, *G. Haraeanum* (*G. asiaticum*), and not of the branch form, *G. japonicum*, as was supposed.

OCCURRENCE IN AMERICA

Clinton (1) reported the occurrence in 1911 of *Gymnosporangium japonicum* on imported plants of *Juniperus chinensis* in Connecticut. He also found the two forms on stems and leaves and followed Shirai in considering them identical. Long (5), after a study of Clinton's material, called attention to the difference between the two forms and described the leaf form as *G. chinense*, considering it distinct from *G.*

[1] See "Literature cited," p. 1009.

Haraeanum. Clinton (2) later admitted that he confused two species, but believed Long not justified in describing the leaf form as new and considered *G. chinense* Long as synonymous with *G. Haraeanum*.

The branch form, *G. japonicum*, has recently (May 19, 1915) been collected on the campus of the University of Washington, at Seattle, Wash., by Dr. J. W. Hotson, and a specimen of it is in the herbarium of Dr. J. C. Arthur and has been examined by the writer.

OCCURRENCE IN OREGON

In the spring of 1915 (Mar. 29) the writer again visited the locality from which he had previously collected the material of *Roestelia koreaensis*. Within 20 feet of the two Japanese pear trees which had shown the infection the previous season and about midway between them two trees of *Juniperus chinensis* were found which showed abundant infection on the leaves of a telial stage of a species of Gymnosporangium. This was determined as *G. Haraeanum*. At the time the collection was made most of the sori had become swollen into gelatinous masses of characteristic shape (Pl. LXXVIII, fig. 3), though a few were found which had not become expanded (Pl. LXXVIII, fig. 2). No other species of Gymnosporangium was found in the vicinity, and no evidence of a branch form was noted.

A considerable quantity of this material was taken to the laboratory of the Department of Botany and Plant Pathology at the Oregon Agricultural College and used in greenhouse infection experiments. No plants of *Pourthiaea villosa* were available, but four potted plants of *Pyrus sinensis* and one each of *Pyrus communis* and *Cydonia vulgaris* were used in the experiments.

The method used was that of suspending branches of the infected juniper over the trees and covering them with large bell jars. This was done on March 30. These were left over the trees for four days, during which time the jars were removed for a few moments daily and the foliage and the inside of the jars sprayed with water. The plants were left covered longer than was intended, it having been the original plan to leave them covered only two days. At the time they were removed it was noted that evidence of infection was already visible on the foliage of the Japanese pear trees. Three or four days later it was evident that pycnia were developing in great abundance on the foliage of these and a few on the quince. There was evidence of initial infection on the trees of *Pyrus communis*, but no pycnia ever developed; only minute black spots finally resulted.

Fully developed æcia were collected from the infected trees of *Pyrus sinensis* (Pl. LXXIX, fig. 1) and *Cydonia vulgaris* (Pl. LXXIX, fig. 2) on June 3, though they were mature fully three weeks earlier. The resulting æcia were found to agree in all respects with the æcia collected in the field the previous year and with descriptions of *Roestelia koreaensis*.

These results, the writer believes, confirm the opinion regarding genetic relationships expressed by Ito and the culture work of Miyabe and Yamada and of Hara, referred to above. They also serve as additional evidence that Shirai's successful infections were obtained with the leaf form rather than with the branch form.

So far as the writer is aware, this is the first record of the complete establishment of any introduced species of Gymnosporangium in this country, though incomplete evidence of the establishment of the same species in California was brought to his attention through a specimen of *Roestelia koreaensis* found in the Arthur herbarium and collected on *Pyrus sinensis* at Oakland, Cal., July 1, 1913, and communicated by Prof. H. S. Fawcett, of the California Experiment Station. Correspondence with Prof. Fawcett and Prof. W. T. Horne, also of the California Experiment Station, revealed that the specimens came from a nursery conducted by Japanese, and that among other things various oriental evergreens were grown. The pears were said to have been originally imported from France in the dormant condition. The presence of this fungus on the leaves of the pears under the conditions is proof that the telial stage must have occurred on some species of Juniperus in the immediate vicinity, though no observations or collections were made. It is evident from this that the rust was at least temporarily established in California at that time.

TAXONOMIC CONSIDERATION

Based upon the results of the infection experiments discussed above, together with the evidence presented in the literature and such studies as the writer has been able to make with the material available in the Arthur herbarium, the present status of the species under discussion is believed to be as follows:

Gymnosporangium koreaense (P. Henn.), n. comb.

Roestelia koreaensis P. Henn., 1899, *in* Warburg, Monsunia, v. 1, p. 5.
Tremella koreaensis Arth., 1901, *in* Proc. Ind. Acad. Sci., 1900, p. 136.
Gymnosporangium asiaticum Miyabe, 1903, *in* Bot. Mag. [Tokyo], v. 17, no. 192, p. (34). (hyponym)
Gymnosporangium Haraeanum Syd., 1912, *in* Ann. Mycol., v. 10, no. 4, p. 405.
Gymnosporangium chinense Long, 1914, *in* Jour. Agr. Research, v. 1, no. 4, p. 353.

Pycnia and æcia on Pomaceae: *Cydonia vulgaris* Pers., reported from Japan and cultured by Miyabe and Yamada; and from Oregon, cultured on June 3, 1915, by H. S. Jackson. *Cydonia japonica* Pers., reported from Japan and cultured by Miyabe and Yamada. No specimens seen. *Pyrus sinensis*, reported from Korea and Japan. (Part of type of *R. koreaensis*, examined.) Cultured in Japan by Shirai, Miyabe and Yamada, and by Hara. Occurred naturally at Orient, Oreg., on June 11, 1914 (H. S. Jackson), and at Oakland, Cal., on July 1, 1913 (H. S. Fawcett). Cultured at Corvallis, on Oreg., June 3, 1915, by H. S. Jackson.

Telia on Juniperaceae: *Juniperus chinensis*, reported from Japan (part of type of *G. Haraeanum*, examined) and from United States in a nursery at Westville, Conn., on stock just imported from Japan on March 28, 1911, by G. P. Clinton (type of *G. chinense*, examined), and from Orient, Oreg., on March 29, 1915, by H. S. Jackson.

Gymnosporangium asiaticum Miyabe is included here on the authority of Ito (4). Regarding *G. chinense*, the writer, after comparing portions of the original collection of this with a specimen of the type collection of *G. Haraeanum*, is inclined to agree with Clinton (2) that they should not be separated. Long (5) gives us the most important basis for separating *G. chinense* from *G. Haraeanum*, the presence of a single apical pore in the upper cells of the former species, found rarely in the thick-walled form, but more commonly in the thin-walled form. He states that in the latter there are two pores in the upper cells always occurring near the septum. A careful examination of a portion of the original collection of *G. chinense* in the Arthur herbarium shows that apical pores occur rarely, even in the thin-walled form, and in every case observed there was a second pore near the septum. The same condition was observed in the type material of *G. Haraeanum*, though rarely. The collection of the writer, made in Oregon, also shows the same condition, but with the apical pores more abundant in the thick-walled form. In all of the collections examined spores were occasionally found in which one of the pores in the upper cell occurred at or near the septum and the other at a point from one-third to one-half the distance from base to apex. The other differences mentioned by Long are largely, the writer believes, due to variation and are not sufficient to justify separation.

Gymnosporangium photiniae (P. Henn.) Kern, 1911, *in* Bul. N. Y. Bot. Gard., v. 7, no. 26, p. 443.

Roestelia photiniae P. Henn., 1894, *in* Hedwigia, Bd. 33, Heft 4, p. 231.
Gymnosporangium japonicum Syd., 1899, *in* Hedwigia, Beibl., Bd. 38, No. 3, p. (141).

Pycnia and æcia on Pomaceae: *Pourthiaea villosa* reported from Japan, cultured successfully by Ito.

Telia on Juniperaceae: *Juniperus chinensis*, reported from Japan and from United States in a nursery at Westville, Conn., on stock just imported from Japan, March 28, 1911, by G. P. Clinton, and at Seattle, Wash., May 19, 1915, by J. W. Hotson.

ECONOMIC IMPORTANCE

Little is known concerning the economic status of the species under discussion. It may be said, however, that any fungus introduced from a foreign land is an unknown quantity and should be treated with suspicion until its status has been established. Several of the American species of Gymnosporangium are already of considerable economic importance, notably *G. juniperi-virginianae* Schw. in the eastern United States and *G. Blasdaleanum* (D. and H.) Kern in the Pacific States.

Gymnosporangium koreaense has been shown to have its æcial stage on the cultivated quince and the Japanese pear. While attempts to infect *Pyrus communis* were unsuccessful, it should be pointed out that only a single attempt was made and it is reasonable to expect that certain varieties of pears, particularly those derived directly or by hybridi-

zation from the oriental species, would be susceptible to infection. It is not known whether this species is capable of infecting the apple. No records of its occurrence on that host have come to our attention.

While the only telial host known for either species is the Oriental juniper, it should be noted that this species is a very variable form, of which many varieties are recognized, and is closely related to several American species of the Sabina group. It is not at all impossible that either of the rusts under discussion might find a congenial host among some of the American species of Juniperus and become firmly established in this way.

The infection experiments of the writer with *Gymnosporangium koreaense* have shown that it develops very vigorously on the quince. Since the species of Gymnosporangium which are known to infect the quince do not usually develop so vigorously on that host as on others, the vigorous growth of this species on the quince may be an indication that *G. koreaense* is rather cosmopolitan in its habits and in a new habitat finally may prove capable of infecting a wide range of pomaceous hosts.

Several of the forms of *Juniperus chinensis* are commonly planted for ornament in various parts of the country, and practically all of these are imported directly from Japan. Both *Gymnosporangium photiniae* and *G. koreaense* are apparently common in Japan and, as shown by the American records, are liable to be frequently introduced on the telial host. If infected trees should be planted in the immediate vicinity of pomaceous hosts capable of harboring the æcial stage, it is possible for either species to become established, as has occurred in Oregon. In the case of the outbreak of *G. koreaense* in the nursery at Oakland, Cal., it is probable that the junipers which were the source of infection for the rust on the pears have been sold and distributed, and the rust may already be established in one or more localities that have not yet come to the attention of plant pathologists.

In the case of *Gymnosporangium photiniae* it is uncertain whether the telial stage is perennial or biennial. Clinton (1) records that an infected tree planted in the greenhouse developed after two years a new sorus in a different part of the stem than the point of original infection. It is known that several other related species which cause fusiform enlargements of the stem are perennial and take more than one season for the development of the telia after infection. As in all species of Gymnosporangium, the infection of the telial host occurs in the summer, and the mature sori do not develop till the following spring or, in some species, until the second spring after infection. *G. koreaense*, so far as known, is an annual form, requiring a new infection of the telial host each year.

In the case of either species it would be difficult to detect the presence of infection during the summer or dormant season, making inspection at the port of entry difficult. To be certain that infected junipers were

not planted, it would be necessary to hold all imported plants in quarantine until the following spring at least, in order to detect the presence of *G. koreaense* and until the second spring for the detection of *G. photiniae*. All trees found diseased should be destroyed, and in case the rust becomes established in any locality it would be advisable to remove the telial host.

LITERATURE CITED

(1) CLINTON, G. P.
1913. Notes on plant diseases of Connecticut. *In* Conn. Agr. Exp. Sta. Ann. Rpt. 1912, pt. 5, p. 341–358, pl. 17–20.

(2) ——
1914. Notes on plant diseases of Connecticut. *In* Conn. Agr. Exp. Sta. Ann. Rpt. 1914, pt. 1, p. 1–29.

(3) HARA, K.
1913. [Gymnosporangium.] *In* Bot. Mag. [Tokyo], v. 27, no. 319, p. (348).

(4) ITO, Seiya.
1913. Kleine Notizen über parasitische Pilze Japans. *In* Bot. Mag. [Tokyo], v. 27, no. 323, p. 217–223.

(5) LONG, W. H.
1914. An undescribed species of Gymnosporangium from Japan. *In* Jour. Agr. Research, v. 1, no. 4, p. 353–356.

(6) MIYABE, K.
1903. [On a species of Gymnosporangium found in Japan.] *In* Bot. Mag. [Tokyo], v. 17, no. 192, p. (34).

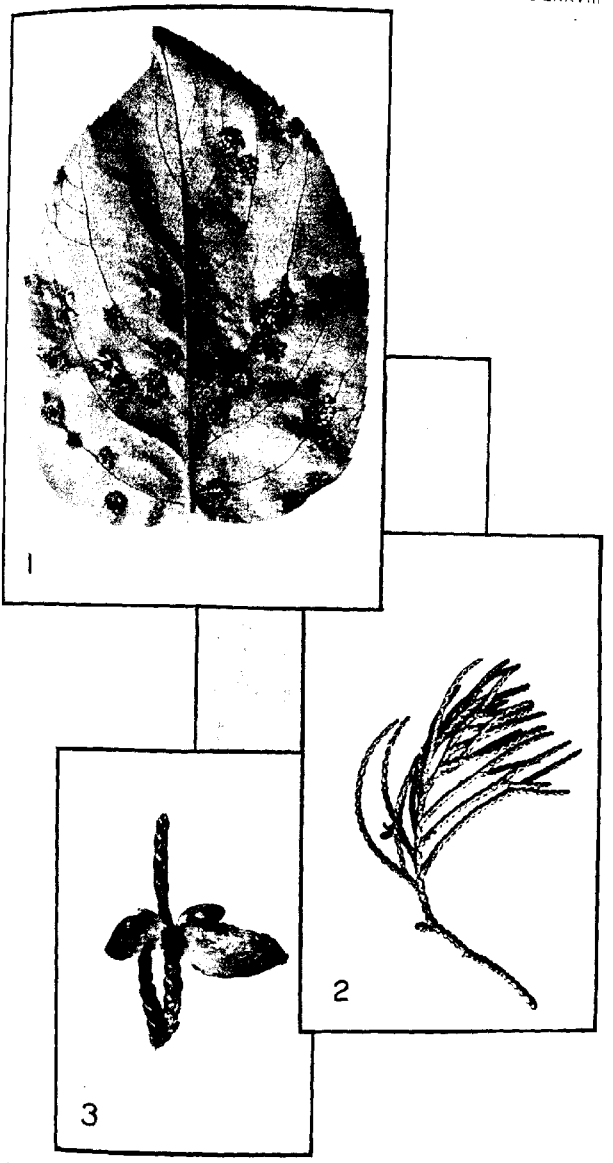
(7) SHIRAI, M.
1900. Über den genetischen Zusammenhang zwischen Roestelia koreaensis P. Henn. und Gymnosporangium japonicum Sydow. *In* Ztschr. Pflanzenkrank., Bd. 10, Heft 1, p. 1–5, pl. 1–2.

PLATE LXXVIII

Fig. 1.—Æcial stage of *Gymnosporangium koreaense* on under surface of leaf of *Pyrus sinensis*. Field collection at Orient, Oreg. Natural size.

Fig. 2.—Telial stage of *G. koreaense* on young twigs of *Juniperus chinensis*. Sori not distended. Field collection at Orient, Oreg. Natural size.

Fig. 3.—Same as figure 2, with sori distended. ×2.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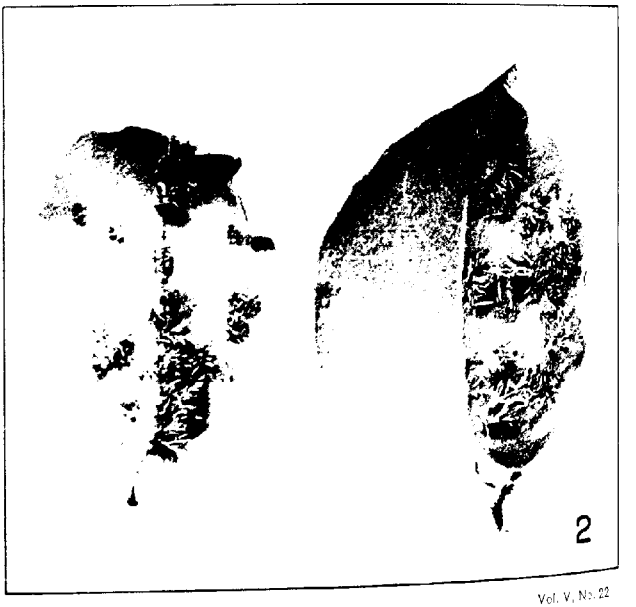
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PLATE LXXIX

Fig. 1.—*Gymnosporangium koreaense* on leaves, petioles, and stems of *Pyrus sinensis*. The result of infection experiments using germinating telia on *Juniperus chinensis*. Natural size.

Fig. 2.—*G. koreaense* on *Cydonia vulgaris*. Natural size.

RELATION OF STOMATAL MOVEMENT TO INFECTION BY CERCOSPORA BETICOLA[1]

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INTRODUCTION

Leafspot infection of the sugar beet (*Beta vulgaris* L.) caused by *Cercospora beticola* Sacc. has been found to be closely related to if not directly controlled by stomatal movement in so far as the host is concerned. Penetration of the leaf by this parasite is effected, so far as known at present, only through open stomata. Consequently the factors favorable to stomatal pore opening become of fundamental importance in the occurrence of the disease.

The factors considered in this paper as most important in influencing stomatal movement are leaf maturity and certain environmental conditions. The term "leaf maturity" as employed in this paper is used to designate the condition of those leaves which have reached a maximum degree of physiological efficiency per unit area. Neither the size of the leaf nor its relative age in days can be taken as a reliable index to its degree of maturity. Under certain conditions young heart leaves of the sugar beet may be stimulated into physiological maturity before they have arrived at the average adult size, and such leaves will always remain small, while leaves which have attained average adult dimensions may still be physiologically immature. The varying degrees of leaf maturity have been found to be accurately indicated by the relative size and number of stomata per square millimeter of leaf surface, and these morphological factors have been observed to remain constant for a given maturity, even though the leaf size and position might indicate another stage of development. The stomata on leaves determined as mature by this method exhibited the greatest movement and responded most readily to changes in the environment. Light may be considered the essentially fundamental external factor affecting stomatal movement, although its influence may be greatly modified by different temperatures and relative humidities, the two factors that will be considered in detail in this paper.

In addition to stomatal movement, infection is also influenced by the rapidity of growth of the conidial germ tube and the maturity of the leaves. Detailed field observations have shown that heart and extremely

[1] This study has been carried on in connection with a detailed investigation of the sugar-beet leafspot conducted by the United States Department of Agriculture in cooperation with a beet-sugar company at Rocky Ford, Colo., during 1912 and 1913. A continuation of the entire problem was made possible during the season of 1914 at Madison, Wis., through the kindness of Dr. L. R. Jones, of the University of Wisconsin.

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young leaves are not susceptible to infection, and that young mature leaves are only slightly so, while mature leaves show the greatest susceptibility. It has also been found that old leaves past their maximum development have for the most part lost their susceptibility, for they seldom show an increase in the number of leaf spots present. Thus the greatest susceptibility to infection becomes concomitant with the greatest stomatal movement, as they both occur on the leaves of the same degree of maturity.

With the varied host and environmental factors favorable, as might be indicated by the stomata on mature leaves remaining open for a period of from five to eight day hours and with vigorous viable conidia of the fungus present, infection would be practically assured.

FACTORS INFLUENCING STOMATAL MOVEMENT

LEAF MATURITY

A study of the stomata on leaves of different maturities has indicated certain specific characters that might be used to determine the comparative development of different leaves. The number of stomata per square millimeter of leaf surface and the stomatal pore lengths have been found to give a good indication of leaf maturity as determined by the size, condition, and position of a leaf on a normal plant. By using the stomatal numbers and pore lengths as a means of measurement, the degree of maturity of any leaf on a heavily infected or otherwise abnormal plant may be determined, regardless of the degree of development indicated by its size and position. This becomes of especial value in the study of the leaves on a plant heavily infected by *Cercospora beticola*, for the young leaves may be mature, though their size and position would indicate immaturity.

Lloyd's[1] (7) method[2] for observing stomata in situ has been used throughout the study in determining the stomatal numbers and pore openings. Microscopic examinations were made near the middle of the blade of leaves which were taken directly from the plants to the stage of the microscope. Readings were continued not longer than two minutes, the stomata remaining unchanged during that time.

On a normally developed sugar-beet plant, pronounced differences are usually found to exist between the central, or heart, leaves, those occupying a midway position on the plant (here designated as mature leaves) and those occurring at the extreme outer portions of the leaf growth (old leaves). On leaves growing in such relative positions read-

[1] Reference is made by number to "Literature cited," p. 1038.

[2] Lloyd's stomatoscope (shown in Pl. LXXX, fig. 1), which was devised later, was kindly lent by the inventor for the studies which were made in Colorado in 1913. Two characters of this instrument, which make it exceedingly valuable for leaf study, are the long stage and the modified condenser, which serves also as a cooling chamber. The instrument also has a basal screw for tripod attachment. In a letter to the authors he has suggested (1) that the objective should be corrected for use without a cover glass, (2) that the focus of the condenser should be capable of being placed 5 mm. above the stage level for proper use in the case of thick leaves, and (3) that smoked glasses should be provided to shield the eyes.

ings were made of the stomatal numbers and pore lengths, together with the leaf size. These readings were taken during the same period and under comparable environmental conditions and the results are given in Tables I, II, and III, each leaf having been given the same number in all the tables.

STOMATAL NUMBERS

It is shown in the general averages of Tabie I that the number of stomata per square millimeter of heart-leaf surface (289.8, upper surface; 353.5, lower surface) is more than 2½ times that on mature leaves (100.7, upper; 130.6, lower), as would be expected. There are in turn more on the mature than on the old leaves (80.1 and 105), while cotyledons have the fewest of all (54.7 and 73.2). The plants studied were grown in the field at Madison, Wis., under favorable conditions, and at the time the readings were made they appeared normal in every way. The older plants were about 7 weeks old, and those from which the cotyledons were studied were 3 weeks old. The cotyledons were green and turgid, comparing in maturity and activity probably with those leaves termed "mature." It may also be noted in the averages that more stomata were present on the lower surface of the leaves than on the upper and that the ratio between the two remained uniform.

TABLE I.—*Average number of stomata on the upper and the lower leaf surfaces of heart, mature, and old leaves and cotyledons of the sugar beet. Readings*[1] *taken at Madison, Wis., on July 6, 1914. The number of readings made per leaf is given in parentheses following each average*

Leaf No.	Heart leaves.		Mature leaves.		Old leaves.		Cotyledons.	
	Upper.	Lower.	Upper.	Lower.	Upper.	Lower.	Upper.	Lower.
2	.....	.....	92.9 (3)	141.1 (2)	.....	.....	69.7 (4)	54.7 (3)
3	*240.7*[2] (2)	*282.2* (3)	94.6 (4)	124.5 (4)	*53.1* (4)	*78.0* (4)	66.4 (4)	92.9 (5)
4	293.8 (4)	325.0 (3)	94.6 (7)	126.1 (5)	59.7 (6)	102.9 (4)	53.1 (4)	78.0 (4)
5	275.5 (3)	391.7 (3)	104.5 (3)	132.8 (2)	71.3 (3)	86.3 (5)	59.7 (3)	59.7 (3)
6	298.8 (3)	373.5 (2)	*124.5* (5)	129.4 (5)	74.7 (2)	99.6 (3)	*76.3* (3)	.....
7	*353.5* (3)	370.1 (3)	92.9 (3)	*99.6* (3)	94.6 (4)	116.2 (3)	66.4 (4)	109.5 (3)
8	298.8 (1)	381.8 (2)	104.5 (3)	126.1 (3)	83.0 (2)	104.5 (3)	74.7 (2)	107.9 (4)
9	315.4 (1)	381.8 (1)	104.5 (3)	141.1 (2)	89.6 (4)	104.5 (3)	*33.2* (3)	*38.1* (3)
10	307.1 (2)	348.6 (2)	99.6 (3)	121.1 (3)	92.9 (3)	126.1 (3)	49.8 (3)	76.3 (3)
11	320.3 (3)	370.1 (3)	109.5 (3)	137.7 (3)	*99.6* (3)	*132.8* (3)	49.8 (3)	91.3 (4)
12	253.9 (3)	308.7 (3)	104.5 (3)	*154.3* (3)	83.0 (1)	99.6 (1)	38.1 (3)	43.1 (3)
13	.....	.....	.....	.....	.....	.....	49.8 (4)	56.4 (5)
14	303.7 (3)	*398.4* (1)	102.9 (4)	127.8 (4)	.....	.....	58.1 (2)	*126.1* (3)
15	.....	.....	99.6 (2)	.....	.....	.....	49.8 (4)	38.1 (3)
16	.....	.....	99.6 (2)	116.2 (1)	.....	.....	.....	.....
17	249.0 (1)	323.7 (2)	*91.3* (2)	149.4 (1)	.....	.....	66.4 (3)	83.0 (1)
19	.....	.....	91.3 (2)	132.8 (2)	.....	.....	41.5	.....
20	.....	.....	.....	.....	.....	.....	33.2 (2)	49.8 (2)
21	257.3 (2)	340.3 (2)	.....	.....	.....	.....	49.8 (1)	66.4 (1)
Average	289.8	353.5	100.7	130.6	80.1	105.0	54.7	73.2

[1] These leaves were used for the readings given in Tables II, III, and V, and each leaf has the same number in all the tables.

[2] Numbers in italics indicate the maximum and minimum variation.

STOMATAL PORE LENGTHS

The stomatal pore lengths of the different types of leaves show variations that are comparable to those observed in stomatal numbers—i. e., a smaller stomatal size must accompany the greater stomatal numbers per unit area. The pore lengths (Table II) of the stomata on the heart leaves (14μ, upper surface; 14μ, lower surface) are on the average about half that of those on the mature leaves (28.5μ, upper, 27.1μ, lower), and in turn the mature leaves show a slightly shorter pore length than those on the old leaves (31.06μ, upper, and 30.5μ, lower) or cotyledons (31.8μ, upper, and 32.1μ, lower), the last two sets being about equal.

TABLE II.—*Average lengths (in microns) of stomatal pores on the upper and the lower leaf surfaces of heart, mature, and old leaves and cotyledons of the sugar beet. Readings*[1] *taken at Madison, Wis., on July 6, 1914. The number of readings made per leaf is given in parentheses following each average*

Leaf No.	Heart leaves.		Mature leaves.		Old leaves.		Cotyledons.	
	Upper.	Lower.	Upper.	Lower.	Upper.	Lower.	Upper.	Lower.
1			33.9 (2)					
2			33.9 (3)	27.5 (2)			32.1 (3)	36.6 (5)
3	12.2 (6)	10.5 (6)	29.6 (12)	26.2 (6)	33.9 (6)	33.9 (4)	33.0 (8)	28.3 (9)
4	12.7 (8)	14.0 (8)	25.8 (7)	26.7 (3)	30.9 (6)	29.6 (6)	33.9 (6)	32.5 (7)
5	11.8 (5)	13.1 (6)	28.3 (7)	25.4 (4)	29.6 (5)	29.6 (6)	29.6 (6)	39.8 (7)
6	18.6 (5)	15.6 (4)	32.5 (7)	26.2 (5)	31.3 (7)	30.0 (5)	40.2 (6)	
7	15.6 (7)	12.7 (5)	29.6 (9)	26.2 (4)	29.6 (3)	29.6 (4)	38.9 (5)	38.9 (8)
8	12.7 (5)	15.2 (5)	27.5 (7)	27.9 (6)			28.3 (7)	29.6 (6)
9	8.8 (7)	8.8 (6)	26.7 (3)	23.7 (6)			36.2 (3)	39.4 (3)
10	10.5 (4)	8.4 (4)	29.6 (6)	27.5 (4)			29.6 (4)	29.6 (5)
11	16.1 (5)	21.2 (4)	29.6 (12)	29.6 (5)			29.6 (5)	29.6 (4)
12	17.7 (5)	16.1 (5)	29.6 (4)	28.8 (6)			29.6 (4)	29.6 (4)
13							29.6 (6)	29.6 (8)
14	16.9 (4)	16.9 (3)	26.7 (6)	25.8 (7)			30.4 (5)	30.0 (6)
15			28.8 (6)				29.6 (6)	27.5 (6)
16			23.7 (6)	29.6 (5)				
17	14.8 (5)	14.8 (4)	27.5 (4)	27.5 (7)			29.6 (7)	29.6 (4)
18			27.5 (5)	27.5 (4)				
19			25.8 (9)	27.5 (9)			29.6(12)	31.3 (9)
21			25.4 (3)	27.5 (4)				
Average	14.0	14.0	28.5	27.1	31.06	30.5	31.8	32.1

[1] These leaves were used for the readings given in Tables I, III, and V, and each leaf has the same number in all the tables.

It thus appears that a definite relation exists between stomatal pore length and maturity of the leaf, although at times a shorter pore length might indicate the maturity as being somewhat less than would be shown by the number of stomata present. This may be due to the completed growth of the epidermal cells being attained before metabolic activity reaches its maximum, and consequently the stomatal pore length would be less.

SIZE AND MATURITY OF LEAF

The sizes of the leaves from which the stomatal numbers and pore lengths have been taken show a difference that is characteristic of comparatively young plants during the early summer. As these plants increased in size, the oldest leaves would for a period be normally much smaller than the mature leaves, since the old leaves had been formed at a time when the plants were small. This difference in size is shown in Table III, where the mature leaves are much larger (18.3 by 15.1 cm.) than the old leaves (10.9 by 7.2 cm.), which in turn are only slightly larger than the heart leaves (9.9 by 6.6 cm.). Since the plants had not yet attained their maximum size, these heart leaves would, when mature, probably be larger even than the present mature leaves. Finally, however, a point would be reached where the mature leaves formed would not be increasingly larger with advanced age of the plants, at which time the mature and old leaves should be approximately the same size. It thus appears that there are great variations throughout the season in the sizes of the leaves that are developed at different periods or under abnormal conditions, owing to disease, unfavorable soil factors, etc. However, leaf maturity, regardless of leaf size, may be determined by the number of stomata per unit area and their pore lengths.

TABLE III.—*Comparative sizes (in centimeters) of heart, mature, and old leaves and cotyledons of the sugar beet. Readings [1] taken at Madison, Wis., on July 6, 1914*

Leaf No.	Heart leaves.		Mature leaves.		Old leaves.		Cotyledons	
	Length.	Width.	Length.	Width.	Length.	Width.	Length.	Width.
1	.....	.....	18	17	.....	.....	.....	.....
2	.....	.....	18	17	.....	.....	.....	.....
3	10	6	11	16	10.5	7	2.5	0.7
4	14	9	21	16	8.5	7	2.3	.7
5	10	16	20	16	10	7.5	3.0	.8
6	10	5	20	16	17	10	2.0	.7
7	10	5	10.5	7.5	11	7	2.5	.7
8	8	6.5	20	16	8	4.5	3.0	.8
9	8	6.5	20	16	10	5.5	3.0	1.0
10	8	6.5	20	16	10	7	2.0	.6
11	10	3.5	20	16	12	8	2.5	.6
12	12.5	7	20	16	12	8	2.5	.8
14	8.5	4.5	20	16	.....	.....	2.4	.6
15	.....	.....	.....	.....	.....	.....	3.5	.8
16	.....	.....	18	15	.....	.....	.....	.....
17	12.5	6	18	13	.....	.....	2.5	8
18	.....	.....	18	15	.....	.....	.....	.....
19	.....	.....	18	13	.....	.....	3.5	1.2
20	.....	.....	.....	.....	.....	.....	3.5	1.0
21	8	4	.....	.....	.....	.....	3.0	1.0
Average	9.9	6.6	18.3	15.1	10.9	7.2	2.7	.8

[1] These leaves were used for the readings given in Tables I, II, and V, and each leaf has the same number in all the tables.

COMPARISON OF FACTORS FOR DIFFERENT REGIONS

A comparison of the observations of stomatal numbers and pore lengths, leaf size and maturity at different times and places and under various conditions indicates the constancy of existing relations. These studies have been made in the field in Wisconsin and Colorado and in the department greenhouse at Washington, D. C. (Table IV). In general, the sizes of leaves are not comparable as read from these three places in that the periods of observation were varied and the controlling factors were different. However, the variations in the number and size of the stomata on the different leaves in a given locality have remained uniform in all readings.

The heart leaves, as would be expected, always exhibited more stomata per unit area and had shorter pore lengths than the mature leaves on the same plant, and, in turn, the mature leaves showed more stomata per unit area than the old mature leaves. It is to be noted, however, that heart leaves in Wisconsin, although comparing them with those studied in Colorado in stomatal pore lengths, showed twice as many stomata per unit area, indicating less maturity and consequently a greater possible ultimate development in area of leaf surface. This difference probably was due in great measure to the almost constant presence of leafspot on the plants observed in Colorado and the great freedom from it in the Wisconsin field from which the data were taken. The accumulative effect of the disease on the plant would be shown by the development of smaller sized leaves with a lessened number of stomata per unit area, showing that they were maturing at a size below normal.

TABLE IV.—*Comparison of the average size of leaf, stomatal numbers, and pore lengths on different leaves of sugar-beet plants studied in Wisconsin, Colorado, and Washington, D. C.*

Locality and leaf maturity	Size of leaf.		Number of stomata per square millimeter of leaf surface.		Stomatal pore length.		Number of leaves in averages.
	Length.	Width.	Upper.	Lower.	Upper.	Lower.	
	Cm.	*Cm.*			μ	μ	
Wisconsin:[1]							
Heart	9.9	6.6	289.8	353.5	14.0	14.0	13
Mature	18.3	15.1	100.7	130.6	28.5	27.1	16
Old mature	10.9	7.2	80.1	105.0	31.1	30.5	10
Cotyledons	2.7	.8	54.7	73.2	31.8	32.1	18
Colorado:[2]							
Old heart	10.2	12.1	144.9	206.2	.....	.....	6
Old heart, uninfected[3]	11.8	8.6	145.9	187.5	14.4	14.8	11
Young mature, infected[3]	13.5	10.3	105.9	142.8	17.8	17.6	13
Mature	16	14.4	80.4	109.6	19.4	18.1	26
Washington, D. C.:[4]							18
Old heart	5.3	3.1	161.0	.....	.....	.....	56
Mature	6.7	4	98.0	.....	.....	.....	57
Old mature	6.9	4.2	74.5	.....	.....	.....	

[1] The results given are the averages taken from Tables I, II, and III.
[2] Readings made in the field from June to August, inclusive, 1913.
[3] The results given are the averages taken from Table X.
[4] Readings made during January, 1914, on potted plants about 8 weeks old grown in the greenhouse.

Mature leaves from Colorado have approximately the same number of stomata per unit area as old mature leaves from Wisconsin, although the stomatal pore lengths are less in the former than in the latter. This would seem to be due in part to the fact that the stomata read in Colorado were not open as widely as those read in Wisconsin, and thus their maximum pore length would not be attained when observed. However, the stomata which were well open in Colorado often had a pore length equal to the average in Wisconsin. The Wisconsin records include the readings made only early in the season on one day under favorable environmental conditions when the stomata were generally wide open. On the other hand, the Colorado records include readings made on various days throughout the season and often under unfavorable environmental conditions when the stomata were only slightly open, and thus they exhibited a short pore length. In such a case the stomatal numbers offer a safer criterion of leaf maturity than the stomatal pore lengths.

The number of stomata per unit area were also read on leaves from a normal mother beet plant growing in the field at Madison, Wis., on July 30, 1914, and the results obtained were entirely comparable to those from the first-year beets, in that leaf maturity could be indicated by the same stomatal numbers. The increase in number of stomata from the oldest, or basal, leaves to those occurring near the tips of the stalks, or the younger leaves, is shown in the following tabulation:

Length of leaf.	Width of leaf.	Average number of stomata per square millimeter of upper leaf surface.	Number of readings.
Cm.	*Cm.*		
20	17	107.9	2
9	5	121.2	3
9	5	137.8	3
6	3.5	187.6	3
4.5	2	204.2	3
3	1.3	240.7	2

LEAF MATURITY AND STOMATAL MOVEMENT

Observations made at different times and on many plants have shown that the degree of stomatal movement is greatly influenced by leaf maturity. In the detailed tests reported, the readings of the stomatal pore widths on leaves of different maturities were made in the field at Madison, Wis., on a day when the sunlight was fairly strong and constant, the temperatures comparatively high, and the relative humidities above 60 per cent (fig. 1). This combination of factors was favorable for stomatal opening, as will be shown later under "Environmental factors." The leaves used in this test were the same as those from which the stomatal numbers and pore lengths have been given in Tables I, II, and III.

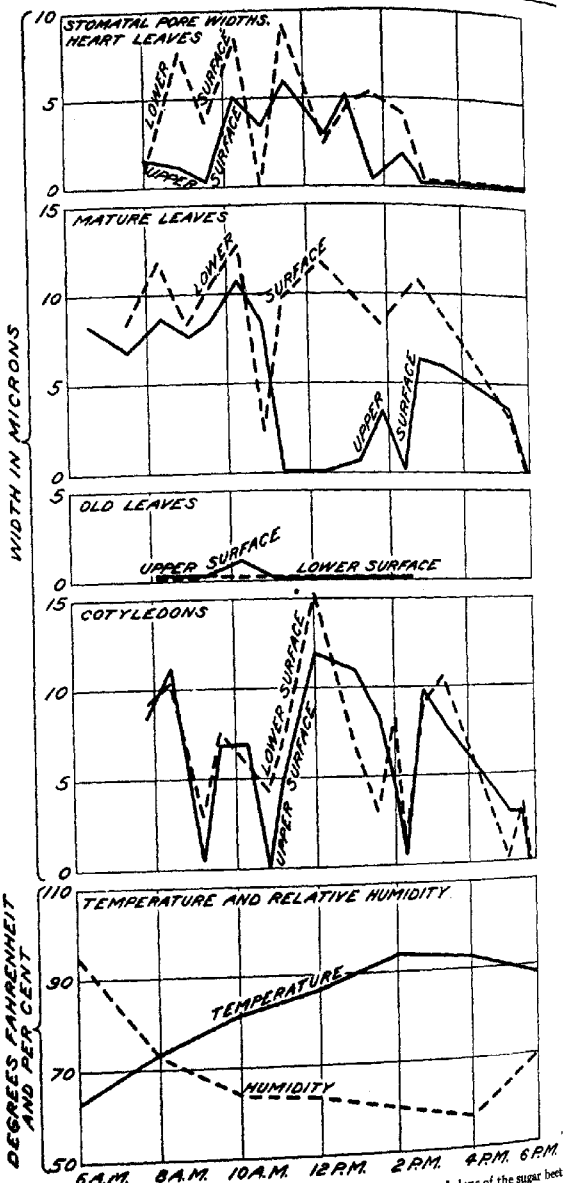

FIG. 1.—Stomatal pore widths on heart, mature, and old leaves and cotyledons of the sugar beet

The results (Table V and fig. 1) show that the widths of the stomatal pores on cotyledons and mature leaves were greater than those on the heart leaves. In general, the stomata on the cotyledons and the lower surface of the mature leaves remained open throughout the day, while those on the heart leaves were entirely closed at 3 p. m. Those on the upper surface of the mature leaves showed a tendency to close from 11 a. m. to 1 p. m., and then to reopen before their final closure at 6 p. m. Shreve (8) found the stomata of *Parkinsonia microphylla* to exhibit this same tendency, since they closed partly during midday and reopened again during the afternoon. The stomata on the old leaves exhibited only slight movement and that on the upper leaf surface from 9 to 11 a. m. Readings were not made early enough in the day to determine the time of initial opening, but the curves indicate that the stomata on the heart leaves opened later than those on the mature leaves and cotyledons. This is shown in figure 1, in that at 8 a. m. the stomatal pore width on the heart leaves was very much less than on the mature leaves and cotyledons, being not more than 2μ on the heart leaves as compared to about 9μ on the others. On cotyledons the stomatal openings on the upper and the lower leaf surfaces remained quite comparable throughout the day. On the mature and heart leaves, however, the stomata of the lower surfaces exceeded in width of pores those of the upper surface. This relation was found to occur almost constantly throughout the day. In all cases the stomata on the upper surfaces closed at about the same time as those on the lower surfaces.

TABLE V.—*Effect of leaf maturity on average stomatal pore widths on the upper and lower leaf surfaces of the sugar beet. Readings*[1] *were taken at Madison, Wis., on July 6, 1914. The number of readings made per leaf is given in parentheses following each average*

Leaf No.	Heart leaves.			Mature leaves.			Old leaves.			Cotyledons.			Time of reading.	Temperature.	Humidity.
	Time of reading.	Upper.	Lower.	Time of reading.	Upper.	Lower.	Time of reading.	Upper.	Lower.	Time of reading.	Upper.	Lower.			
	a. m.	μ	μ	*a. m.*	μ	μ	*a. m.*	μ	μ	*a. m.*	μ	μ	*a. m.*	°*F.*	
1	.....	.....	.....	6. 30	8.4 (2)	.....	.....	.....	.....	.....	.....	.....	6. 00	62	95
2	.....	.....	.....	7. 30	6.7 (3)	8. 4 (2)	.....	.....	.....	7. 50	8.4 (3)	9.2 (5)	.....	.....	.....
3	8. 00	2.5 (6)	0.3 (6)	8. 15	7.6 (12)	11.8 (6)	8. 05	0.4 (6)	0.0 (4)	8. 25	11.3 (8)	10.1 (9)	8. 00	74	73
4	8. 50	2.1 (8)	7.6 (8)	9. 00	7.6 (7)	8.4 (3)	8. 50	.4 (6)	.0 (6)	9. 10	.4 (6)	2.9 (7)	.....	.....	.....
5	9. 30	.1 (5)	3.8 (6)	9. 35	8.4 (7)	10.5 (4)	9. 25	.3 (5)	.4 (6)	9. 40	6.7 (6)	7.1 (7)	.....	.....	.....
6	10. 15	5.0 (5)	8.4 (4)	10. 15	10.9 (7)	12.7 (5)	10. 10	1.3 (8)	.0 (5)	10. 20	6.7 (6)	.....	10. 00	82	64
7	10. 35	3.4 (7)	.0 (5)	10. 50	8.4 (9)	2.5 (4)	10. 55	.0 (3)	.0 (4)	10. 50	.0 (5)	4.2 (8)	.....	.....	.....
8	11. 30	5.9 (5)	9.2 (5)	11. 20	.0 (7)	9.7 (6)	11. 25	.0 (5)	.0 (5)	11. 15	5.0 (7)	7.1 (6)	.....	.....	.....
	p. m.			*p. m.*			*p. m.*			*p. m.*			*p. m.*		
9	12. 30	2.9 (7)	2.5 (6)	12. 15	.0 (3)	11.8 (6)	12. 25	.0 (6)	.0 (6)	12. 00	12.7 (3)	15.2 (3)	12. 00	87	63
10	1. 05	5.0 (4)	4.6 (4)	1. 10	.8 (6)	9.7 (4)	1. 20	.0 (4)	.0 (4)	1. 00	10.9 (4)	6.7 (5)	.....	.....	.....
11	1. 45	.3 (5)	6.3 (4)	1. 50	3.4 (12)	8.4 (5)	1. 55	.0 (5)	.0 (5)	1. 35	8.4 (5)	2.9 (4)	.....	.....	.....
12	2. 25	1.7 (5)	4.2 (5)	2. 20	.0 (5)	9.7 (6)	2. 25	.0 (6)	.0 (5)	2. 00	4.6 (4)	8.4 (4)	2. 00	94	60
13	.....	.....	.....	.....	.....	.....	.....	.....	.....	2. 15	.3 (6)	.4 (8)	.....	.....	.....
14	2. 55	.0 (5)	.2 (3)	.....	.....	.....	.....	.....	.....	2. 25	.3 (6)	.4 (8)	.....	.....	.....
15	.....	.....	.....	2. 50	6.3 (6)	2.6 (7)	.....	.....	.....	2. 45	9.7 (5)	9.2 (6)	.....	.....	.....
16	3. 30	.0 (5)	.0 (5)	3. 25	5.9 (6)	.....	.....	.....	.....	3. 15	8.0 (6)	10.5 (6)	4. 00	93	57
17	.....	.....	.....	5. 25	5.2 (8)	4.6 (10)	.....	.....	.....	5. 15	2.9 (7)	.0 (4)	.....	.....	.....
18	.....	.....	.....	5. 50	3.4 (9)	6.3 (9)	.....	.....	.....	5. 40	2.4 (12)	3.1 (9)	.....	.....	.....
19	6. 00	.0 (5)	.0 (5)	.....	.....	.....	.....	.....	.....	5. 50	.0 (5)	.0 (6)	.....	.....	.....
				6. 00	.3 (3)	.0 (4)	.....	.....	.....	6. 15	.0 (5)	.0 (5)	6. 00	88	70

[1] These leaves were used for the readings given in Tables I, II, and III, and each leaf has the same number in all the tables.

This, then, would indicate that the stomata on old leaves exhibit very little movement; that those on heart leaves open, but not so widely as on mature leaves, and close earlier; that on cotyledons and mature leaves they open widely, indicating their great activity. Therefore, in the study of the environmental factors influencing stomatal movement only mature leaves have been considered, since they were always available and responded readily to changes in environment. They also represent that portion of leaf growth which is most susceptible to infection by *Cercospora beticola*. If it is true, as claimed by Iljin (4) and others, that variation in the osmotic pressure of the guard cells regulates stomatal movement, then it might be concluded that the leaves which exhibit the greatest stomatal movement are also the most active metabolically and are consequently the most important to plant development.

ENVIRONMENTAL FACTORS

It is generally agreed by various investigators that the chief external factors influencing stomatal movement are light and temperature, while a difference of opinion exists as to the influence of relative humidity. Some believe that humidity greatly affects the degree of stomatal opening, while others consider it of only minor importance. Wilson and Greenman (12) found that the stomata on plants of *Melilotus alba* which were left covered with a glass case, thus being in a nearly saturated atmosphere, were well open, while on those which were left standing in the drier open air the stomata were nearly all closed. Darwin (2) gave evidence to prove that stomata were very sensitive to changes in the humidity, closing on being taken from a high to a low humidity and opening under the reverse conditions when all the plants were exposed to approximately the same light. According to Lloyd (6) "there is a small amount of evidence that a high relative humidity favors, as a condition, the wider opening of the stomata in the ocotillo" and in regard to *Mentha piperita*, also a desert plant, he concludes ". . . in these plants, that as long as wilting does not take place a low relative humidity does not reduce the stomatal opening."

As shown by the present study, the writers believe that, while light may be considered a fundamental factor in stomatal movement, yet stomatal closure is effected by low relative humidity, even though light is active. The relative humidity present at any time, together with an optimum temperature, has been found to be a good criterion of the amount of stomatal movement that may be possible under the existing conditions.

LIGHT

In this study no attempt has been made to determine the exact relation of light to stomatal movement. Only a few scattered readings have been made to determine what effect direct sunlight has on stomatal

opening (Table VI), and the results agree, in general, with those obtained by Lloyd (6) with desert plants. When the entire leaf was exposed to sunshine, as when the leaf blade stood parallel to the sun's rays, the stomata showed the same or a greater pore opening on the lower than on the upper leaf surface (series A). This was also found to be true with leaves entirely in the shade (series B). When the sun struck vertically upon the leaf blade, an accelerating effect on stomatal opening usually resulted, regardless of which surface was exposed to the sun (series C and D). This is also in agreement with the work of Balls (1, p. 231), in which he found that the stomata on the cotton plant opened widely in the sunlight and closed partly in the shade. The leaves in series C, read on July 18, indicate a point noticed by Lloyd (7) that the stomata near the apex of a leaf might have less pore width than those near the base, "a condition readily understandable if wilting is progressive from the apex of the leaf downward."

TABLE VI.—*Effect of sunshine and shade on the width of the stomatal pore opening of the sugar-beet plant at Rocky Ford, Colo., in 1913*

Date.	Hour.	Relative humidity.	Temperature.	Stomatal pore width.	
				Upper surface.	Lower surface.
			°F.	μ	μ
SERIES A (ENTIRE LEAF IN SUN)					
May 17	7.15 a. m.	58	67	1.8 (3)	1.8 (4)
Aug. 4	7.30 a. m.	77	68	6 (6)	[a] 7.8 (7)
SERIES B (ENTIRE LEAF IN SHADE)					
May 17	7.15 a. m.	58	67	0 (6)	0 (6)
June 2	7.45 a. m.	100	65	1.5 (8)	[b] 6.3 (8)
June 3	9.30 a. m.	71	69	0	0
Aug. 4	7.30 a. m.	77	68	1.3 (7)	4.7 (5)
SERIES C (UPPER LEAF SURFACE IN SUN; LOWER IN SHADE)					
May 24	7.30 a. m.	69	68	4.2 (4)	8.6 (5)
May 26	1.45 p. m.	51	91	9.4 (6)	7.6 (4)
May 27	8.00 a. m.	62	78	4.5 (4)	0 (6)
July 18	9.00 a. m.	91	72	[c] 5.7 (3) [d] 7.2 (3)	[c] 5.7 (3) [d] 6.5 (3)
Aug. 4	7.30 a. m.	77	68	5.7 (4)	0 (5)
SERIES D (UPPER LEAF SURFACE IN SHADE; LOWER IN SUN)					
May 19	8.00 a. m.	65	63	[e] 3.8 (7)	[f] 10.8 (6)
May 24	7.30 a. m.	69	68	2.5 (5)	3.4 (5)
May 26	1.45 p. m.	51	91	6.5 (6)	9.4 (5)
May 27	8.00 a. m.	62	78	1.0 (6)	7.4 (6)

[a] Wet from dew.
[b] All wide open.
[c] Apex.
[d] Base.
[e] Many closed.
[f] All open.

TEMPERATURE AND RELATIVE HUMIDITY

The determination of the effect of varied temperature and relative humidity on the opening of the stomatal pore of the sugar-beet plant was made under conditions which were somewhat under control. The plants used for study were first-year beets about 3 months old and of thrifty growth which had been grown in a deep soil bed in the greenhouse at Rocky Ford, Colo. A good root development was thus made possible, and normal leaf production had been accomplished. The leaves used for the readings were all mature and averaged about 14 cm. wide and 20 cm. long. Direct readings of the widths of the stomatal pores were made on plants both left free in the greenhouse and kept covered during the time of the experiment with a large glass humidity box (Pl. LXXX, fig. 2) of about 20 cubic feet capacity. This box was five-sided and could be placed over plants in a manner comparable to the bell-jar method. Aeration was made possible by this means and room was also available for a hygrothermograph, so that constant-humidity and temperature records were available without any disturbance of the plants. Comparable hygrothermograph records were also kept among the leaves freely exposed in the greenhouse and both instruments were checked by means of a cog psychrometer (Pl. LXXX, fig. 2). Middle-blade portions of different leaves were taken from all plants and stomatal readings made by the "in situ" method. The definite data of the experiments conducted on May 16, 17, and 20 and June 3 are given in Table VII and the graphic representations in figures 2 to 5.

TABLE VII.—*Effect of varied temperature and relative humidity on stomatal pore opening on sugar-beet leaves at Rocky Ford, Colo., in 1913. Comparable readings were taken in the greenhouse on plants covered by a large glass humidity box and on those left freely exposed to ordinary greenhouse conditions*

Date and time of reading.	In humidity box.				In greenhouse.			
	Temperature.	Relative humidity.	Average stomatal pore widths.[a]		Temperature.	Relative humidity.	Average stomatal pore widths.[a]	
			Upper leaf surface.	Lower leaf surface.			Upper leaf surface.	Lower leaf surface.
	° *F.*	*Per ct.*	μ	μ	° *F.*	*Per ct.*	μ	μ
May 16:[b]							1.8 (3)	0 (4)
9.00 a. m.	68	70	9.0 (5)	7.9 (5)	77	43	0 (5)	0 (5)
1.30 p. m.	92	46	12.6 (4)	10 (4)	90	16	0 (5)	0 (5)
4.15 p. m.	89	54	8.6 (5)	0 (4)	93	18	0 (7)	0 (5)
7.00 p. m.	71	79	0 (5)	.36 (4)	75	24.5		
May 17:[c]							0 (5)	0 (5)
5.00 a. m.	51	95	.36 (6)	2.1 (12)	52	73.5	1.8 (3)	1.8 (5)
7.15 a. m.	60	67	6.8 (6)	2.7 (4)	67	58	2.5 (9)	2.1 (4)
8.30 a. m.	63	66	7.3 (7)	8.2 (9)	72	50	5.4 (4)	5.4 (5)
10.00 a. m.	73	65	6.8 (4)	7.5 (4)	78	38	1.8 (6)	5.4 (5)
11.00 a. m.	80	63	7.2 (6)	9 (5)	83	31	7.2 (3)	6.8 (4)
1.30 p. m.	79	60	7.2 (4)	7.2 (4)	80	32	0 (4)	0 (5)
4.20 p. m.	70	74	7.2 (3)	6.4 (3)	71	34		

[a] The number of readings is given in parentheses following each average.
[b] The sun shone brightly throughout the entire day.
[c] The sun shone brightly up to 4 p. m.

TABLE VII.—*Effect of varied temperature and relative humidity on stomal pore opening on sugar-beet leaves at Rocky Ford, Colo., in 1913*—Continued

Date and time of reading.	In humidity box.				In greenhouse.			
	Temperature.	Relative humidity.	Average stomatal pore widths.		Temperature.	Relative humidity.	Average stomatal pore widths.	
			Upper leaf surface.	Lower leaf surface.			Upper leaf surface.	Lower leaf surface.
May 20:[a]	°F.	Per ct.	μ	μ	°F.	Per ct.	μ	μ
5.00 a. m.	50	95	0.3 (6)	0.4 (13)	51.5	93	0 (7)	0.3 (9)
6.00 a. m.	51	95	.7 (8)	1.8 (9)	52	91	0.25 (11)	.2 (10)
7.00 a. m.	53	94	.3 (7)	1.6 (7)	54	83	.14 (10)	.28 (10)
8.00 a. m.	56	85	3.24 (7)	5 (6)	61	65	2.8 (6)	1.4 (8)
8.30 a. m.	63	76	2.16 (6)	2.8 (6)	63	64	2.1 (8)	2.1 (7)
9.00 a. m.	64	75	5.7 (6)	7.2 (5)	65	59	3.9 (6)	4.3 (6)
9.30 a. m.	64	75	6.1 (9)	7.2 (9)	65	59	4.6 (7)	3.8 (6)
10.30 a. m.	65	66	5.7 (6)	5.7 (4)	67	53	5 (6)	7.5 (7)
11.00 a. m.	68	65	7.2 (6)	7.2 (6)	68	57	1.8 (6)	1.4 (7)
11.45 a. m.	71	66	7.2 (4)	7.2 (4)	72	53	2.1 (6)	0 (5)
1.30 p. m.	75	57	9 (4)	11 (5)	74	45	1.08 (5)	1.08 (6)
2.15 p. m.	75	58	7.2 (6)	7.5 (5)	74	42	3.2 (7)	1.6 (6)
2.45 p. m.	73	57	7.2 (5)	7.2 (4)	71	42	3.6 (7)	4.06 (6)
3.30 p. m.	74	53	5.7 (5)	3.2 (5)	71	42	1.5 (5)	0 (5)
4.00 p. m.	75	53	6.8 (5)	6.1 (6)	74	40	2.1 (5)	0 (5)
June 3:								
7.45 a. m.	67	100	3.6 (3)	4.3 (4)	69	69	1.8 (4)	1.08 (4)
9.00 a. m.	70	100	6.3 (4)	7.38 (4)	67	64	2.5 (5)	.14 (5)
9.30 a. m.	72	100	7.5 (4)	7.2 (4)	69	71	2.8 (4)	0 (5)
10.00 a. m.	74	100	7.5 (4)	7.4 (4)	75	68	4.4 (5)	2.5 (5)
10.15 a. m.	80	100	7.2 (4)	7.3 (5)	73	75	6.1 (5)	6.1 (5)
10.30 a. m.	82	100	6.4 (4)	5.8 (4)	73	67	7.2 (4)	6.8 (5)
11.45 a. m.	93	100	9.3 (4)	9.3 (3)	79	62	4.5 (4)	9.1 (5)
12.15 a. m.	94	97	7.8 (4)	8.5 (4)	82	63	9.4 (3)	8.1 (3)
1.30 p. m.	96	93	7.02 (6)	7.5 (5)	80	56	3.8 (10)	2.7 (9)
2.00 p. m.	94	95	9.4 (6)	9.4 (6)	80	58.5	1.6 (11)	2.3 (12)
2 30 p. m.	85	95	7.8 (5)	7.8 (4)	75	57	5.8 (6)	0 (8)
3.00 p. m.	89	100	5.4 (6)	6.4 (6)	75	57	0 (5)	0 (5)
3.30 p. m.	75	100	5.4 (6)	4.3 (5)	75	57	0 (5)	0 (6)

[a] Intermittent clouds and sunshine up to 11.45 a. m., then bright sunshine until 2.25 p. m.; cloudy to 3.30 p. m., and then sunshine for the rest of the day.

Usually the temperature in the humidity box was practically the same as that outside in the greenhouse at the same time. Although no definite study has been made to determine the temperature most favorable to stomatal movement, it is to be noted that good stomatal opening occurred between 8 a. m. and 5 p. m., and during that time the temperature increased, on the average, from about 65° to 85° F. and decreased to 80° F. Only on June 3 was the temperature in the humidity box much higher than that outside in the greenhouse, and it appears that neither of these temperatures (96° in the humidity box and 80° in the greenhouse at 1.30 p. m.) produced a change in the degree of stomatal opening.

On the other hand, the humidity in the two places was quite different, being always higher inside than outside of the humidity box. To this difference in humidity the marked variation in the pore opening of the stomata has been attributed. For example, on May 16 the humidity ranged about 30 units higher inside than outside of the box (fig. 2), and the stomata were well open in the former place and closed practically throughout the day in the latter. On the upper leaf surface in the greenhouse only slight opening occurred at 9 a. m. and this disappeared

by 1.30 p. m. During this time the relative humidity fell from 43 to 16 while inside the humidity box it ranged from 70 to 46 and the average width of the pores of the stomata increased from 9 to 12.6μ. The stomata on the upper leaf surface were also open wider and remained open longer than those on the lower, while all were closed by 7 p. m. These points

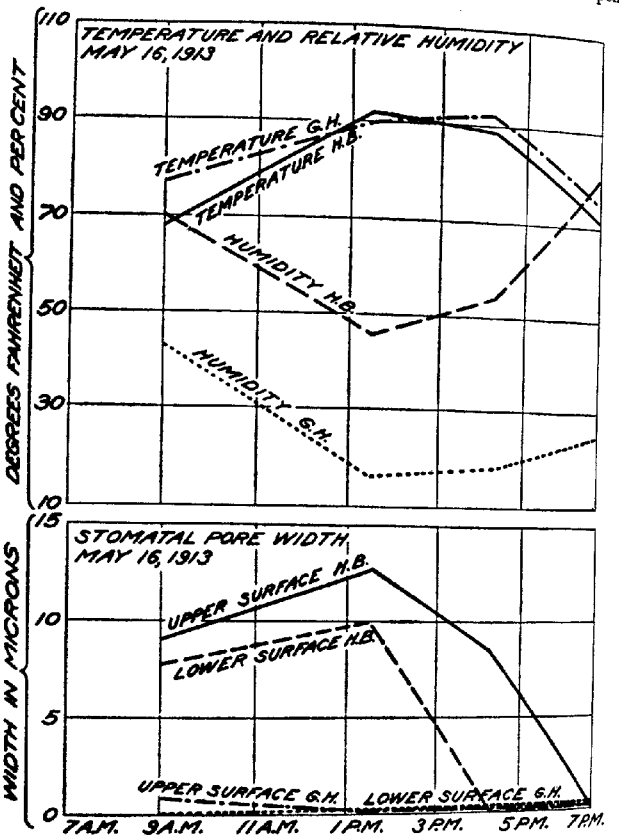

FIG. 2.—Stomatal pore widths on mature leaves kept under different relative humidities in a humidity box (H. B.) and free in the greenhouse (G. H.) at Rocky Ford, Colo., on May 16, 1913 (Table VII).

seem to indicate that the relative humidity as supported by soil moisture, transpiration, etc., must remain, in general, above a certain percentage in order that the maximum influence of light may be realized. Otherwise, if the humidity is too low, the light factor becomes in some way less operative, and the stomata open to a less extent and close earlier.

In another test made on the following day, the humidity ranged from 9 to 40 units higher inside the humidity box than in the greenhouse (fig. 3), and throughout the day the stomata were open wider in the former place than in the latter. At 5 a. m. all the stomata were closed except those on the lower leaf surface in the humidity box, which were slightly open. In general, the initial opening probably occurred soon after 5 a. m., for at 7.15 a. m. the stomata were all open, those in the humidity box being open wider than those outside. This point opposes the theory that the stomata in the humidity box remain well open during midday on account of the less intense light due to the additional window-

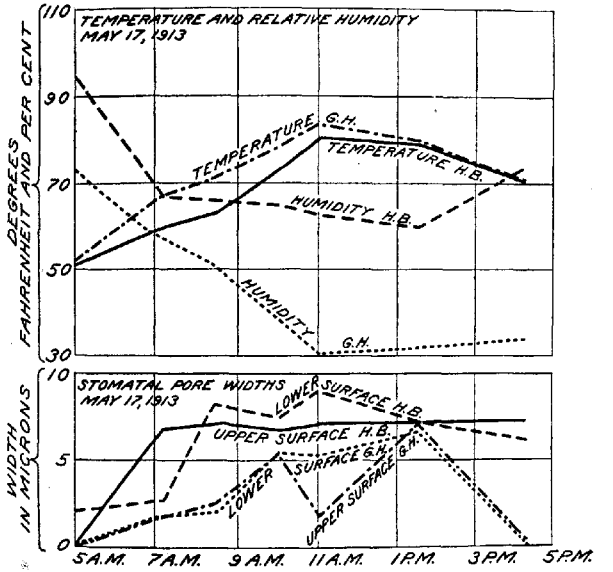

FIG. 3.—Stomatal pore widths on mature leaves kept under different relative humidities in a humidity box (H. B.) and free in the greenhouse (G. H.) at Rocky Ford, Colo., on May 17, 1913 (Table VII).

glass covering, while during the same period, those outside the humidity box close as a reaction to the more intense unobstructed light. If this were true, then, the stomata in the humidity box would open later in the day than those outside, because the light in the former place would be weaker. As a matter of fact, the stomata in the humidity box opened earlier and had greater pore width than those outside, even when thus exposed to the weaker light. The conclusion that may be drawn from this is that the relative humidity is the indicative factor of the causes which produce this difference. It should be noted that in the humidity box the humidity did not fall below 60 during the day, and the stomata were still open at 4.20 p. m., when the last reading for the day was made.

Outside in the greenhouse the humidity ranged from 31 to 34 after 11 a. m., and the stomata were entirely closed at 4.20 p. m.

A comparison of the stomatal pore widths of the leaves in the greenhouse on May 16 with those in the same place on May 17 shows that on the former day the stomata were practically closed all day, while on the latter they opened early and remained fairly well open till after 2 p. m. The humidity on the two days was quite different, being appreciably higher on the 17th than on the 16th. This offers an explanation for the difference in stomatal pore opening, though, of course, conditions on the two separate days can not be compared too closely.

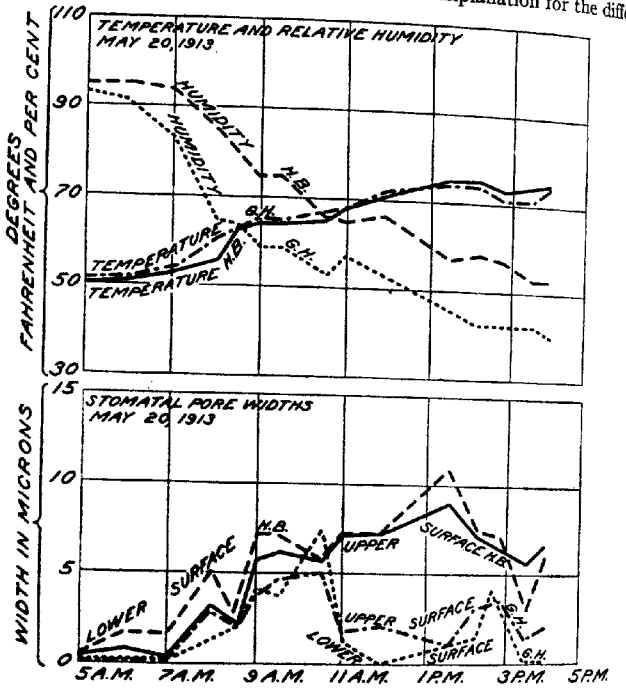

FIG. 4.—Stomatal pore widths on mature leaves kept under different relative humidities in a humidity box (H. B.) and free in the greenhouse (G. H.) at Rocky Ford, Colo., on May 20, 1913 (Table VII).

In another test, made on May 20, the stomata in the humidity box again showed greater widths of pores than those outside in the greenhouse (fig. 4) and the humidity ranged about 10 units higher throughout the day in the former place than in the latter. The greatest difference in the stomatal opening in the two places occurred after 11 a. m. when the stomata in the humidity box had much greater stomatal pore widths than those outside. The humidity remained generally near or above 60 in the box, while outside it was, on the average, below 50. The initial

opening in both places occurred about 5 a. m., and in the humidity box the opening on the lower leaf surface exceeded that on the upper, this relation remaining uniform throughout the day. This tendency is also indicated in figure 3 in the greater stomatal opening of the lower over the upper leaf surface in the humidity box. These observations in general agree with the findings of other investigators. Darwin (2) found that the stomata on the lower surface often opened earlier and remained open longer than those on the upper, though this was not always true. He believed that the difference in the opening was due to illumination rather than to any inherent distinction between the stomata. Livingston and Estabrook (5) found in the study of the stomata on several different

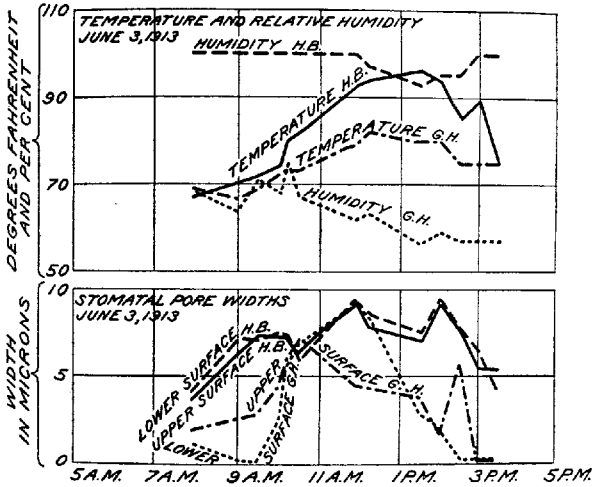

FIG. 5.—Stomatal pore widths on mature leaves kept under different relative humidities in a humidity box (H. B.) and free in the greenhouse (G. H.) at Rocky Ford, Colo., on June 3, 1913 (Table VII).

kinds of plants that those on the upper surface open and close more rapidly and close more completely than those on the lower. Lloyd (7) observed with cotton that—

> The initial opening on September 30, 1911, occurred about 6.30 a. m., from which hour on a progressive opening movement was followed, the stomata of the lower surfaces opening somewhat in advance of those of the upper, though some exceptions to this appear.

Again, on June 3, after all the beds in the greenhouse had been watered on the preceding evening and the humidity box placed at that time over a portion of the plants for the test, the same general results were obtained, in that the stomata opened wider and remained open longer in the humidity box with higher humidity active for a longer period than in the green house (fig. 5). During this test the stomata in the greenhouse remained open during midday till about 3 p. m., owing probably to the fact that

the humidity remained comparatively high—above 60. A comparable difference is noted in the humidities and stomatal pore widths taken on this date and on May 20. After 11 a. m. the humidity on June 3 was generally above 60 and the stomata had pore widths of more than 5μ until after 1 p. m., when the opening gradually decreased until closure occurred about 3 p. m. On May 20, after 11 a. m., the humidity was generally slightly above 50 and the stomatal pore opening was reduced from 5μ at 10 a. m. to about 2μ at 11 a. m., after which time it seldom exceeded this amount.

A few readings were made in the field at various times during the season to get an indication of the stomatal movement under such conditions. On June 21 the stomata were found to be well open at 3 p. m. and later at a humidity of 60 or above (Table VIII). On June 23 the stomata were widely open from 8.30 to 10.40 a. m., even though the humidity dropped to as low as 40 at 10.10 a. m. The readings were not continued long enough to determine whether this low humidity would produce stomatal closure during midday. However, the readings taken on July 18 indicate that at 2 p. m. the stomata had a smaller pore width than at any other reading during the day and at that time the lowest humidity (57.5) of the day occurred. Two readings were made at the same time in this field. The one made near the center of the field, where the plants were large and close together, showed the stomata to be open (8.7 upper, 1.8 lower) at a humidity of 57.5, while the other made at the edge of the field, where the plants were small and far apart, showed the stomata to be closed at a humidity of 43.5. The maturity was determined to be the same for both sets of leaves used. In this case the soil-moisture content was noted to be much lower at the edge than in the center of the field, as the low humidity would indicate.

TABLE VIII.—*Stomatal pore openings on leaves of sugar-beet plants growing in the field at Rocky Ford, Colo., in 1913, together with the temperature and relative-humidity records taken among the leaves at that time*

Date and time of readings.	Temperature.	Humidity.	Average stomatal pore widths.[1]	
			Upper leaf surface.	Lower leaf surface.
	°F.		μ	μ
June 21:				
3.00 p. m.	85	60	10.4 (4)	10.08 (5)
3.45 p. m.	.....	.....	4.96 (7)	6.6 (6)
4.30 p. m.	77	65	1.72 (9)	5.1 (7)
June 23:				
8.30 a. m.	74	60	10.8 (3)	9.9 (4)
9.20 a. m.	79	52	13.5 (4)	10.3 (4)
10.10 a. m.	83	39.5	10.6 (7)	7.1 (8)
10.40 a. m.	85	46.5	12.9 (5)	10.8 (3)
July 18:				
9.00 a. m.	72	91	6.3 (6)	6.4 (3)
10.30 a. m.	74	87	4.6 (3)	4.1 (3)
11.15 a. m.	82	67	9 (5)	14.4 (4)
2.00 p. m.[2]	83	57.5	8.7 (8)	1.8 (10)
2.00 p. m.[2]	89	43.5	0 (10)	0 (10)

[1] The number of readings made is given in parentheses following each average.
[2] These readings were taken at two different places in the same field.

Therefore, it may be concluded that if the relative humidity remains above 60 during the hours of daylight the stomata will probably be found open, while with a lower humidity the stomatal opening will decrease until it becomes greatly reduced and with still lower humidity the stomata may usually be found completely closed, or at least as nearly so as ever occurs. In an irrigated area especially, where the humidity is very largely controlled by the soil moisture, a high humidity may be directly due to a high soil-moisture content and would indicate increased plant activity. The beneficial effects of high humidity on increased plant growth is generally recognized. Wollny (13), who grew plants of barley, vetch, alfalfa, flax, and potato under conditions giving three degrees of humidity, found that with an increase in the degree of humidity there was an increase in the production both of the absolute quantity of fresh material and of dry matter. On the other hand, low soil-moisture content would greatly check such activities, and a low humidity, which would be associated with such a condition, would indicate marked differences in stomatal movement. Thus, it appears that a low humidity with its associated causes and effects results in diminished stomatal movement, and then the existing percentage of relative humidity becomes an important and convenient index to stomatal activities.

FACTORS INFLUENCING INFECTION

A consideration of the factors additional to, and somewhat preliminary to, stomatal movement that have been found to influence infection includes some of the conditions that affect both parasite and host in this relation. The effect that media, light, and temperature have on the rapidity of germ-tube growth becomes important in the relation that the fungus bears to leaf penetration. On the other hand, the maturity of the leaf, which controls stomatal mobility, plays a comparable part in this interrelation.

RAPIDITY OF GERM-TUBE GROWTH

No difference has been found to exist in the effect that north light and darkness have on the rapidity of germ-tube growth at a constant temperature. From the data given in Table IX it appears that all conidia germinated and had approximately the same average germ-tube lengths, together with a comparable average number of germinating cells per spore, regardless of the light factor. Consequently, under field conditions conidial germination would be expected to proceed equally fast under night or day conditions, except in direct sunlight, where the heat factor becomes important in causing rapid evaporation.

TABLE IX.—*Effect of light and medium on the germination of conidia of Cercospora beticola, at a temperature of 24° C., on August 12, 1913, at Rocky Ford, Colo.*

Environment.	Number of hours of growth.	Average percentage of germinating conidia.	Average number of cells per conidium.	Average number of germinating cells per conidium.	Average length of germinating tube.
					μ
Distilled water, north light	6¼	100	.....	2.47	43.28
Distilled water, dark room	6½	100	.....	2.4	41.11
Distilled water, north light	8	100	9.42	4.14	56.31
Distilled water, dark room	8½	100	8.69	3.46	65.77
Bean decoction, north light	9	100	9.44	3.33	55.48
Irrigation water, north light	9¾	100	10.16	3.83	91.69
Soil decoction, north light	10	100	6	3.00	98.42

Germination also occurred equally well in distilled water, bean decoction, soil decoction, and irrigation water, showing that a nutrient medium did not hasten germination nor did it retard it. It is also to be noted that the conidia were incubated nearly twice as long in soil decoction as in distilled water, which would account for the longer germ tubes in the soil decoction. In both solutions 100 per cent of the conidia germinated. The condensed moisture that may be found on leaves then would seem to give a favorable medium for conidial germination and that germ-tube growth could take place rapidly in it. It has been found that only a short time is necessary for germination to take place, since newly formed conidia may begin to germinate in three hours after being placed in water cultures at 26° C. The germinating tubes from such conidia may increase 5μ in length in 40 minutes.

The effect of high temperatures on conidial germination is not considered in this discussion. However, in another phase[1] of the study of the sugar-beet leafspot, it has been determined that a period of days with extreme high night (70° F.) and day (104° F.) temperatures together with low relative humidity, a condition that may occur at times in an irrigated region, is inimical to the life of the conidia. This factor then becomes of importance in considering conidial growth and development under natural environment.

LEAF MATURITY

Near the middle of the summer or later, in a sugar-beet field infected generally with leafspot, the individual plant presents a typical picture of the disease. A cluster of uninfected heart and slightly infected young mature leaves occurs at the center of the plant, while all other leaves on the same plant are heavily infected. A comparison was made of the stomata on such heart and young mature leaves, or the oldest uninfected and the youngest infected leaves, on each of several plants. The study

[1] The thermal relations of the fungus will be discussed in a later paper entitled "Relation of climatic conditions to infection by Cercospora beticola."

was carried on in August, 1913, near Rocky Ford, Colo., and the readings of the two types of leaves from the same plant were made near together so that all time factors might, so far as possible, be eliminated. The results show that on the average the number of stomata is less and their pore length is greater (Table X) on the infected leaves than on the uninfected, showing the greater maturity of the former. Some variations in these numbers occur, but it is to be noted that the four infected leaves with the greatest number of spots present have, on the average, fewer stomata per square millimeter of leaf surface and a greater stomatal pore length than the four infected leaves with the least number of spots.

TABLE X.—*Comparative average maturity of Cercospora beticola infected (young mature) and uninfected leaves (heart) of the sugar-beet plant as shown by the number and pore length of the stomata. Readings*[1] *taken on August 5 to 11, 1913, at Rocky Ford, Colo.*

INFECTED YOUNG MATURE LEAVES[2]

Leaf No.	Size of leaf.		Average number of stomata.		Average stomatal pore lengths.		Number of leaf-spots per leaf.
	Length.	Width.	Upper leaf surface.	Lower leaf surface.	Upper leaf surface.	Lower leaf surface.	
	Cm.	*Cm.*			μ	μ	
1	17.5	12.5	98.4 (3)	123 (1)	19 (3)	19 (2)	24
2	17	12.5	68.06(3)	106.6 (3)	19 (5)	19 (3)	21
3	9.5	9	95.1 (3)	111.5 (3)	19 (6)	19 (3)	21
4	14.5	10	102.5 (4)	127.9 (3)	19 (4)	19 (2)	14
5	10	7.5	106.6 (3)	155.8 (2)	19 (6)	17.5 (5)	9
6	10.5	9	110.7 (2)	139.4 (2)	19 (5)	19 (5)	5
7	15	10.5	77.9 (2)	123 (2)	15.2 (6)	19 (6)	5
8	11.5	12	114.8 (2)	137.3 (4)	17.1 (6)	15.2 (2)	3
9	15	12.5	118.9 (2)	164 (2)	15.5 (5)	13.3 (2)	3
10	10.5	8.5	114.8 (2)	172.2 (2)	19 (4)	19.7 (5)	1
11	13.5	9.5	133.9 (3)	183.1 (3)	15.2 (4)	16.7 (7)	1
Average.	13.1	10.3	103.8	140.3	17.8	17.8	.....

UNINFECTED HEART LEAVES[2]

1	14.5	9.5	123 (2)	164 (1)	19 (3)	17.4 (3)	.....
2	14	9.5	118.9 (2)	172.2 (1)	15.9 (5)	15.2 (5)	.....
3	8.5	8	145.2 (3)	147.6 (3)	17.1 (8)	15.2 (5)	.....
4	12.5	8	135.3 (2)	166.4 (3)	13.1 (4)	15.2 (2)	.....
5	13	8	133.9 (3)	184.5 (2)	13.6 (6)	15.2 (4)	.....
6	10.5	7	144.3 (3)	174.6 (3)	15.2 (3)	17.1 (2)	.....
7	13	9.5	131.2 (1)	205 (2)	11.4 (4)	13.3 (6)	.....
8	9	9.5	127.1 (2)	184.4 (2)	13.9 (5)	11.4 (4)	.....
9	13	10	192.7 (2)	225 (2)	13.3 (8)	13.3 (6)	.....
10	9	6.5	192.7 (2)	241.9 (2)	13.3 (4)	15.2 (6)	.....
11	12.5	9.5	161.2 (3)	196.8 (3)	12.1 (7)	14.4 (5)	.....
Average.	11.8	8.6	145.9	187.5	14.4	14.8	.....

[1] The number of readings made per leaf is given in parentheses following each average.
[2] Infected leaf 1 was on the same plant as uninfected leaf 1, infected leaf 2 was on the same plant as uninfected leaf 2, and so on through the series. The leaves of each pair were read at the same time.

The averages for the eight leaves mentioned are:

Leaf No.	Size of leaves.		Number of stomata.		Length of stomatal pores.		Number of leaf-spots.
	Length.	Width.	Upper.	Lower.	Upper.	Lower.	
	Cm.	*Cm.*			*Cm.*	*Cm.*	
1 to 4	14.6	11	91	117.2	19	19	20
8 to 11	12.6	10.6	120.6	164.1	16.7	16.2	2

It is also to be noted that infected leaf 11, which had only one spot, had the shortest average stomatal pore lengths (except leaf 9) and the highest number of stomata per area of any of the infected leaves studied. From these figures it would further appear that of all the uninfected leaves studied, only leaf 1 would have a stomatal count and pore length that would indicate leaf susceptibility. It might be concluded that this leaf remained uninfected merely by chance and that the others were uninfected because they had not as yet reached the maturity which would allow infection to occur.

Detailed field observations made of the amount of infection that appeared on the different leaves of many sugar-beet plants during an entire season have again shown that the greatest number of leafspots developed on the mature leaves. The records from one plant are shown in Table XI. The leaves were tagged and numbered consecutively, beginning with the outermost, or oldest, so that the new leaves tagged on all days after the first one were heart leaves. As these grew older they became susceptible to leafspot, and with increased maturity usually became heavily infected, and finally the death of the leaf occurred. Those leaves, whose numbers are in italic, on the last date reported were killed by the fungus. From 400 to 1,000 spots were sufficient to kill a leaf, depending on its size, in a few days. While the death of many of the leaves not reported as killed by *Cercospora beticola* was no doubt hastened by the presence of the fungus, yet age and other factors were predominating causes of the death of the leaf.

The results obtained show that, as a rule, infection did not take place readily on old yellow leaves, but occurred most readily on active green leaves. It is true that there was often a large increase in the number of spots present on the leaves during the few days just previous to the death of the leaf, as is shown by leaves 21, 24, 25, 27, 35, and others on this one plant (Table XI), but such leaves were not normally old. They were no doubt green and quite active when infection took place and merely died prematurely and very suddenly as a result of the great number of spots produced.

TABLE XI.—*Number of leafspots present on different leaves of varying maturity on one sugar-beet plant in a medium-early field from June 24 to Sept. 19, 1913, at Rocky Ford, Colo.*

Leaf No.	June.		July.													August.																										September.									
	24	28	2	7	9	10	12	14	16	21	23	25	28	30	1	5	7	9	11	13	15	18	22	25	28	30	1	3	6	8	10	13	15	17	19
1	0																																		
2	0																																		
3	0																																		
4	0																																		
5	0	0																																	
6	1	1																																	
7	1	1	1																																
8	0	0	0	0	1	1	1																												
9	0	0	0	0	0	0	0	2	2	2	2	3	3	3	3																				
10	0	0	1	4	5	5	5	5	5																										
11	0	0	0	0	3	3	3	3																											
12	0	0	0	4	4	4	4	4	4	4	4	4																							
13	0	0	0	4	5	5	5	5	5	5	5																								
14		0	0	0	0	0	0	0	0	2	2	2																							
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0																
16			0	0	0	0	0	0	0	0	0	0	0	0	4	4																			
17			0	0	0	0	0	0	0	0	1	1	3	3	8	23	28	38	38																
18				0	0	0	0	0	0	0	0	0	0	1	1	11	24	26	26																
19				0	0	0	0	0	0	0	0	0	2	4	8	21	21	26	26	38	42	50	50	70	300	300	300								
20				0	0	0	0	0	0	0	0	0	0	0	4	6	11	11	15	20	30	40	40	56	350	350	350								
21					0	0	0	0	0	0	0	0	3	3	3	13		13	25	25	27	60	76	115	155	450									
22						0	0	0	0	0	0	0	3	3	3	9	16	20	29	31	31	40	61	85	140	140	140								
23							0	0	0	0	0	0	2	7	12	32	39	50	50																
24								0	0	0	0	0	3	4	5	15	18	18	19	32	49	54	96	150	300	300	400								
25									0	0	0	0	0	0	4	12	15	20	20	26	32	33	46	130	600	600	800	800							
26										0	0	0	0	0	1	2	7	12	25	28	37	43	56	68	110	380	600	600	600							
27											0	0	0	1	1	3	6	9	17	18	19	30	46	70	85	200	300	450	500							
28												0	0	1	6	9	15	17	23	29	49	62	80	100	150	350	600	600							
29													0	0	5	9	14	20	24	24	26	61	70	100	170	300	300							
30													0	0	1	1	4	7	10	18	20	22	50	50	50	70	600	600	600	600				
31													0	0	0	3	3	4	11	13	18	23	45	48	50	95	130	350	450	450	450				
32														0	0	0	0	4	7	9	13	13	13	20	20	38	60	260	480	650	700	800	800	800		
33														0	0	3	3	7	14	19	21	24	47	76	105	220	220	450	500	600	600	600				
34															0	0	0	2	10	16	22	22	30	33	34	55	90	350	480	700	750	750	750	750		
35															0	0	0	5	10	10	14	14	38	52	95	160	165	300	300	350	450					
36															0	0	1	1	7	9	10	16	71	116	165	250	250	350	350	520	520	520				
37															0	0	3	9	9	11	16	22	37	39	53	58	120	180	350	420	450	550	600	600	600	
38															0	0	1	1	1	11	28	58	82	95	121	160	270	400	500	550	600	650	700	900	900	900
39																0	0	0	0	1	4	21	49	90	165	180	500	500	500	500	700	900	900	900	
40																	0	0	0	0	3	11	24	64	112	145	145	230	390	570	570	570	570		
41																		0	0	0	8	14	22	33	44	64	95	380	450	500	500	550	550	550	550	550
42																			0	0	3	3	5	9	16	28	160	300	320	320	350	400	500	500	500	
43																				0	1	13	20	47	112	150	190	260	320	365	600	600	600	600	600	
44																					0	0	1	7	18	41	170	170	245	260	260	260	260	300	300	450
45																						0	0	13	55	76	240	250	650	650	650	650	650	650	650	650
46																						0	0	1	4	19	210	380	440	500	500	550	650	900	900	900
47																							0	0	0	0	39	92	170	240	340	450	450	550	550	550	600
48																							0	0	5	12	170	300	400	400	400	400	400	500	650	650
49																								0	0	5	75	155	157	165	250	290	290	400	400	400	450
50																								0	1	5	60	120	240	240	300	400	500	500	500	500	650
51																								0	0	0	85	150	170	210	250	350	350	450	600	600	750
52																								0	0	1	2	6	7	27	47	120	175	200	240	240
53																									0	26	44	64	104	166	220	300	300	350	580	580
54																									0	4	5	8	10	43	120	170	260	260	260	260
55																										0	0	0	0	2	13	58	85	110	120	140
56																										0	0	0	1	6	9	39	45	64	64	75
57																										0	0	0	3	8	26	34	40	50	70
58																										0	0	0	0	0	0	0	0	3	3
59																											0	0	0	0	0	0	0	0	0
60																											0	0	0	0	0	0	0	1	1
61																												0	0	0	0	0	0	0	0
62																													0	0	0	0	0	0	0
63																														0	0	0	0	0	0
64																																		0	0
65																																			
66																																			

During the first half of the season infection did not take place in the heart and young leaves, but later, as in August, during a heavy attack by the fungus they became infected soon after they were large enough to be tagged. This might be explained by the fact that since many of the

leaves were killed by the fungus, the plants were forced to produce more new leaves in an effort to keep up their normal activities. Under such conditions the new leaves formed, appeared to mature earlier than usual, and never became as large as normal. Thus, they became susceptible to infection by *Cercospora beticola* quite early in their development, and often became infected while comparatively small.

This difference in the susceptibility of the different leaves is shown in a general way in Table XI by the diagonal grouping of the three types of leaves—namely, the very young, the mature, and the old. The upper diagonal indicates either no increase in spots on the old leaves, or a slight increase on those which were still somewhat active. The lower diagonal indicates the very young leaves on which there occurred few or no spots, while the middle section represents the mature, active leaves of the plant on which the greatest increase in infections took place. A great increase in the number of infections developed on either the same leaf (reading to the right) or on the entire plant (reading diagonally) as the season advanced.

The mature leaves therefore show the greatest susceptibility to leafspot infection and possess the characters which allow the freest penetration of the host tissue by the fungus. Such leaves, as previously shown, have on the average a stomatal count on the upper surface of approximately 100 per square millimeter with a stomatal pore length of 28μ and exhibit the greatest stomatal movement. Thus, the greatest susceptibility to infection becomes concomitant with the greatest stomatal movement, for they both occur on the leaves of the same degree of maturity.

STOMATAL MOVEMENT AND GERM-TUBE PENETRATION

It may then be concluded that a favorable daily temperature (70° to 90° F.), combined with a relative humidity which does not fall below 60 at any time, together with daylight, will offer conditions under which the stomata on the mature leaves should remain open throughout the day. This condition of the host associated with favorable growth factors for the parasite would usually allow germ-tube penetration and leafspot development.

With these factors active in producing stomatal opening, detailed studies were made of germ-tube penetration from material that had been collected in the field during controlled tests. For these experiments newly formed conidia from recently developed leaf spots were sprayed on mature sugar-beet leaves about 7 p. m. After an incubation period of 11 days numerous typical leaf spots appeared. Portions of these leaves were taken 24, 36, 48, 60, and 72 hours after inoculation, killed and stained according to modifications [1] of the method given by Vaughan

[1] These modifications were suggested by Miss Pearl M. Smith, of the Botany Department of the University of Wisconsin. After the acetic alcohol had acted for 12 to 24 hours, the material was washed for 6 to 8 hours in 95 per cent alcohol, stained in Flemeze's stain overnight, and destained with acid alcohol until the leaf tissue became a clear red, or even pink in places. The material was washed in 95 per cent alcohol until the acid was removed and mounts made in Euparal. Balsam, as a mounting medium in these studies, was not found to give a good differentiation between the stomata and the penetrating fungous mycelium.

(11). An examination of several hundred slides prepared at different times by this method from inoculated leaves has shown that conidia may germinate, produce long germinating tubes and yet not penetrate closed stomata (fig. 6). On the other hand, wherever penetration was found to occur, the stomata were open, and although it has long been known that this organism gains an entrance through the stomata, this point has never been mentioned. Thümen (9, p. 50–54) seems to have been

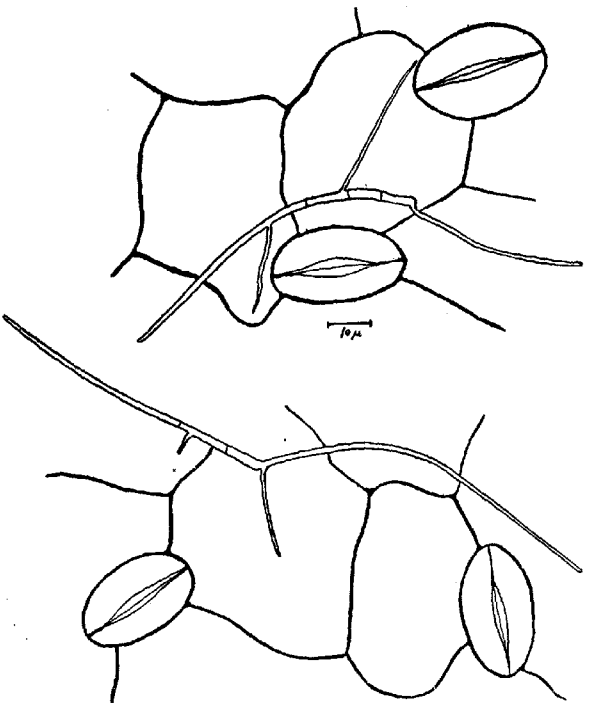

FIG. 6.—*Cercospora beticola:* Conidia germinating on a sugar-beet leaf, but germ tubes not entering or being greatly attracted by closed stomata.

the first to state that a spore which is carried by some means to a green and yet not too old, and thereby hardened, beet leaf, is able to germinate in the shortest time, penetrate into a stoma, and form a number of hyphæ. Frank (3) also agrees with this observation, adding that it is characteristic that the tufts of conidiophores grow out of the stomata. However, no mention seems to be made of the stomatal movement necessary for host penetration.

As soon as penetration of the stoma was gained by the germ tube, a marked change was noted to take place in the character of the fungous growth produced, as indicated by different staining qualities. The conidium and the slender germ tube external to the spore opening stained lightly, while the cells in the pore opening or beneath the stoma stained much more deeply and were comparatively large and round (Pl. LXXXI, *A*, *B*, *F*). It was only rarely observed that penetration into two different stomata took place by germ tubes from one conidium (Pl. LXXXI, *B*, b). In the case observed, the two stomata were near each other and slight germ-tube growth was sufficient for the penetration of both. As a rule, however, only one germinating tube from a conidium has been found to penetrate the host tissue, although it is known that, if this tube does not penetrate before its desiccation takes place, another cell of the conidium may germinate later before the entire conidium loses its viability and penetration might again be possible. At times the pore wall of a guard cell may be penetrated and the growth gradually spread to the adjoining epidermal cells (Pl. LXXXI, *F*, *c*). Normally, however, the germ tubes grow through the pore opening, probably receiving some stimulus from the guard cells and form round, heavily staining mycelial cells which pile up directly in the air chamber below the pore opening. The fungus then grows toward the parenchyma cells (Pl. LXXXI, *C*, *d*) and flatten out against their walls, probably for nutritive purposes. At times, without further development within the host, the fungus grows back out through the stoma and produces conidiophores (Pl. LXXXI, *D*, *e*). In such a case new conidia might be produced before an extensive area of the host tissue had been killed. Usually, however, the fungus grows farther into the host before conidia are formed. It probably is true, as first suggested by Uzel (10), that the fungus causes asphyxiation and consequent collapse of the parenchyma cells, since only a slight intercellular growth of the fungus occurs. An attempt by the host cells to isolate the invading organism is seen in the massing of heavily staining substances (Pl. LXXXI *E*, *f*) in the parenchyma cells which adjoin the air chamber. Under certain conditions this isolation probably is accomplished and the host cells then remain turgid and normal. Where this can not be done, the cells surrounding the fungous mycelium collapse (Pl. LXXXI, *G*), the mycelium gradually produces tufts of conidiophores, and the characteristic leafspot is formed. The host under normal growth conditions is able to isolate this infected area, though as a result of severe, abundant infections, entire leaves may be covered with the conidiophore tufts of the fungus.

It then appears that there is no attractive force existing between the closed stomata and the conidial germ tubes of the fungus, and also that the latter do not possess enzymic power to directly penetrate the epidermal cells. However, with open stomata germ-tube penetration may occur, even though some length must be attained before the tube can

reach the pore opening. The reaction upon penetration induces a great change in the type of fungous growth, the fungous cells becoming large and round. It is to be concluded that since growth continues immediately in the air chamber below the stomata, the stomatal function of gaseous interchange is needed for the development of the mycelium in the host, as well as a force for initial penetration. It seems evident, therefore, that since germ-tube penetration may occur only when the stomata are open, and since stomatal movement is directly related to daylight hours, infection takes place only at this time.

SUMMARY

The study of the relation of stomatal movement to infection of the sugar-beet plant by *Cercospora beticola* Sacc. has revealed that certain morphological and environmental factors influence stomatal activity, and, in turn, the latter, together with a favorable growth of the fungus, influences infection.

Leaf maturity, light, temperature, and relative humidity are factors concerned with stomatal movement.

Leaf maturity may be determined by two characters which for any given stage have been found to remain uniform—i. e., the number of stomata present per square millimeter of leaf surface, and the length of the stomatal pore. These characters, taken together, give a good indication of leaf maturity, regardless of leaf size or position on the plant Leaf maturity has a direct relation to stomatal activity in that movement is greater on mature than on young leaves, while on old leaves only very slight movement has been observed.

Light is probably one of the fundamental environmental factors that influence stomatal movement, and while direct sunlight may have an accelerating action, it is not essential for stomatal opening, since stomata may open widely in the shade.

Good stomatal opening has been obtained at temperatures ranging from 70° to 90° F. With these optimum temperatures active, relative humidity, with its associated causes and their effects, greatly influences stomatal movement. A high humidity favors stomatal opening, while a low humidity is associated with closure of the stomata. If the humidity remains above 60 through the day hours, the stomata will probably remain well open; but if it falls much below 50, stomatal closure will probably result.

Some of the factors influencing infection of beet leaves by *C. beticola* are rapidity of germ-tube growth, maturity of the leaves, and stomatal movement.

Fresh viable conidia of *C. beticola* germinate equally well and grow rapidly in distilled water, soil decoction, irrigation water, and bean decoction, in either darkness or diffused light at 24° C.

Infection, both artificial and natural, occurs best on mature leaves, and this is associated with the movement of the stomata.

Penetration of the leaf by the conidial germ tubes of *C. beticola* has been observed to occur only through *open* stomata, and consequently infection probably takes place during the day hours. An isolation of the invading organism is attempted by the leaf cells as soon as penetration occurs, but when this is not successful, the fungus by further growth produces a well-defined leafspot.

LITERATURE CITED

(1) BALLS, W. L.
1911. Cotton investigations in 1909 and 1910. *In* Cairo Sci. Jour., v. 5, no. 60, p. 221–234, pl. 2.

(2) DARWIN, Francis.
1898. Observations on stomata. *In* Phil. Trans. Roy. Soc. London, s. B, v. 190, p. 531–621.

(3) FRANK, A. B.
1897. Neuere Beobachtungen über die Blattfleckenkrankheit der Rüben (Cercospora beticola). *In* Ztschr. Ver. Rübenzuckerindus. Deut. Reichs, Bd. 47 (n. F. Jahrg. 34), Tech. T., p. 589–597, pl. 8.

(4) ILJIN, W. S.
1914. Die Regulierung der Spaltöffnungen im Zusammenhang mit der Veränderung des osmotischen Druckes. *In* Bot. Centbl. Beihefte, Abt. 1, Bd. 32, Heft 1, p. 15–35, illus.

(5) LIVINGSTON, B. E., and ESTABROOK, A. H.
1912. Observations on the degree of stomatal movement in certain plants. *In* Bul. Torrey Bot. Club, v. 39, no. 1, p. 15–22.

(6) LLOYD, F. E.
1908. The Physiology of Stomata. 142 p., illus., 14 pl. Washington. (Carnegie Inst. Wash. Pub. 82.)

(7) ———
1913. Leaf water and stomatal movement in Gossypium and a method of direct visual observation of stomata in situ. *In* Bul. Torrey Bot. Club, v. 40, no. 1, p. 1–26, illus.

(8) SHREVE, Edith B.
1914. The Daily March of Transpiration in a Desert Perennial. 64 p., illus. Washington. (Carnegie Inst. Wash. Pub. 194.)

(9) THÜMEN, Felix von.
1886. Die Bekämpfung der Pilzkrankheiten unserer Culturgewächse. 157 p. Wien.

(10) UZEL, H.
1905. Ueber den auf der Zuckerrübe parasitisch lebenden Pilz Cercospora beticola Sacc. *In* Ztschr. Zuckerindus. Böhmen, Jahrg 29, Heft 9, p. 501–502, 2 pl.

(11) VAUGHAN, R. E.
1914. A method for the differential staining of fungous and host cells. *In* Ann. Mo. Bot. Gard., v. 1, no. 2, p. 241–242.

(12) WILSON, W. P., and GREENMAN, J. M.
1892. Preliminary observations on the movements of the leaves of Melilotus alba, L. and other plants. *In* Contrib. Bot. Lab. Univ. Penn., v. 1, no. 1, p. 66–72, pl. 9–13.

(13) WOLLNY, Walter.
1898. Untersuchungen über den Einfluss der Luftfeuchtigkeit auf das Wachstum der Pflanzen. 43 p., pl. Halle a. S. Inaugural Dissertation.

PLATE LXXX

Fig. 1.—Stomatoscope designed by Dr. F. E. Lloyd and used for a part of these studies.

Fig. 2.—Humidity box in place over plants in the greenhouse for maintaining different relative humidities. Also a cog psychrometer used for checking hygrothermographs kept among the sugar-beet plants.

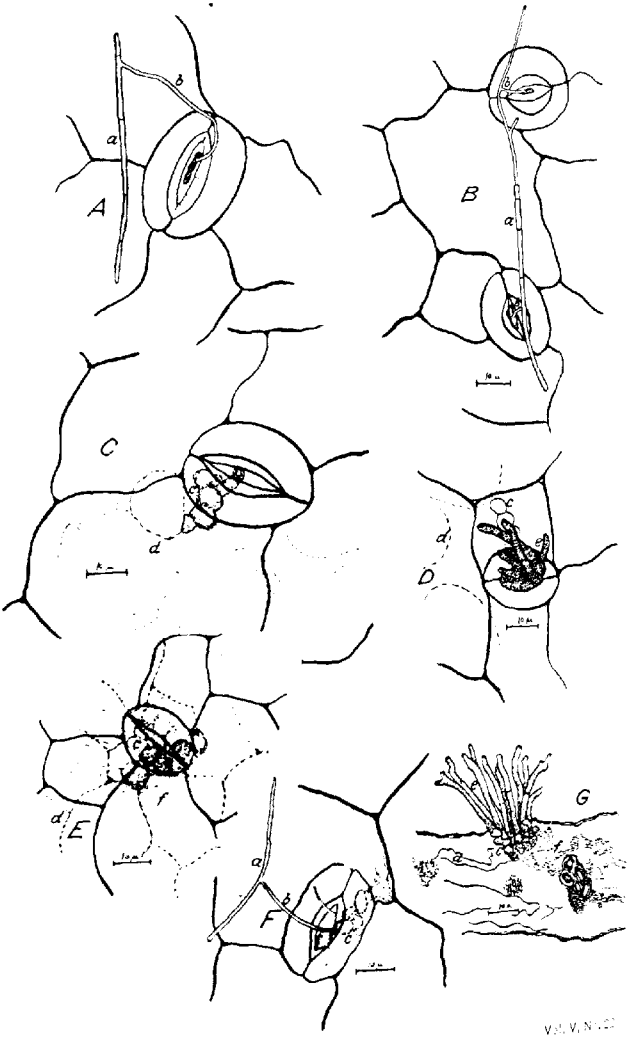
A
B
C
D
E
F
G
a
b
c
d
e
f

PLATE LXXXI

Cercospora beticola Sacc.

Fig. 1.—Conidia germinating on a sugar-beet leaf, with germ tubes entering open stomata. *A*, *a*, conidium; *b*, germ tube. *B*, *a*, conidium; *b*, *b*, two germ tubes penetrating two stomata. *C*, *c*, host mycelium below stoma in air chamber and forming a haustorium against a palisade parenchyma cell (*d*) represented with their chloroplasts by dotted lines. *D*, *c*, host mycelium in air chamber; *d*, parenchyma cells; *e*, exit of conidiophores. *E*, *c*, host mycelium; *d*, parenchyma cells; *f*, heavily staining host substance probably secreted for isolation purposes. *F*, *a*, conidium; *b*, germ tube; *c*, host mycelium in guard cell and epidermal cell. *G*, *c*, host mycelium or sclerotium; *d*, collapsed parenchyma cells; *e*, conidiophores; *f*, heavily staining host substance. (Camera-lucida drawings.)

A METHOD OF CORRECTING FOR SOIL HETEROGENEITY IN VARIETY TESTS[1]

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Men with practical experience in conducting variety tests and fertilizer experiments are free to admit that in many cases the results of ordinary field trials are of little or no value. The reason for this lies in the large number of factors which are beyond the control of the experimenter. In many instances variation in any one of these uncontrollable factors may influence the final results to a greater extent than the one controlled variable for which the experiment was undertaken.

On the other hand, field trials and variety tests play an important part in agricultural investigations. Such tests are an indispensable adjunct to plant-breeding work. The final test of new varieties or new strains must be made under field conditions. It is therefore of the greatest importance that methods should be devised which will in some measure at least take account of these uncontrollable factors.

No one of these factors is of more importance than the variation in the soil in different plots. It is practically impossible to secure for such field trials a tract of land that is absolutely uniform. The literature of variety tests abounds in illustrations of this fact.

In 1897 Larsen (8),[2] on the basis of results with timothy, reached the conclusion that more exact results were obtained where a given area was divided into a large number of plots than when it was divided into a few larger ones.

Holtsmark and Larsen (7) extended this idea and supplied additional evidence. Hall (1) in 1909 and Mercer and Hall (9) and Hall and Russell (2) in 1911 laid great emphasis upon soil heterogeneity in field tests. Among other things they did much to determine the most suitable sizes for experimental plots.

Montgomery (10, 11) has produced evidence showing that systematic repetition of plots over a given area reduces the variability in proportion to the number of repetitions; further, that while increase in the size of a plot decreases the variability up to a certain limit, a further increase in size is not attended by a corresponding decrease in variability.

As a result of these several investigations, it has become evident that much more reliable results are obtained by using several systematically repeated small plots than by using a single large one. This method is rapidly coming into more general use in field tests of all kinds. Never-

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 93.
[2] Reference is made by number to "Literature cited," p. 1050.

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theless, where for various reasons it is impossible to make a large number (10 to 20) of repetitions, the factor of soil heterogeneity still enters into the average yield. One or two exceptionally high or exceptionally low yields will unduly influence the average where the number of repetitions is only four or five.

In a series of papers Harris (3, 4, 5, 6) has called attention to various phases of the experimental error in field tests. In his most recent paper on this subject Harris (6) has proposed a method of measuring the heterogeneity of the soil of a field. The principle employed by Harris is stated thus (432–433):

> If the irregularities in the experimental field are so large as to influence the yield of areas larger than single plots, they will tend to bring about a similarity of adjoining plots, some groups tending to yield higher than the average, others lower.

This tendency to grouping of the high- and low-yielding plots is evident in most field experiments. It is clearly shown in the diagrams published by Montgomery (10).

The measure which Harris proposes for this heterogeneity (or homogeneity) of a field is the correlation between the yield of the ultimate small plots and the yield of various groups of contiguous plots. The more nearly this correlation approaches zero the more homogeneous the field. The more differentiated a given field is in regard to good and poor soil, the greater will be the value of the correlation coefficient.

This method of measuring the heterogeneity of a field is dependent somewhat upon the size of the ultimate plots and also upon the method of grouping. It does, however, mark a distinct advance in our method of dealing with small plot experiments.

While Harris's method provides a *measure* of the substratum heterogeneity in a given field, it does not provide any means of obtaining a corrective term for individual plots. While in field experiments it is of importance to know the amount of heterogeneity in the field as a whole, it is usually of much more importance to obtain some correction to apply to individual plots which will in some measure even up the differences in soil conditions.

The present paper is the result of an attempt to obtain such a corrective term. It is realized that the method proposed is far from ideal. It is believed, however, that it marks a step in this direction, and it is hoped that it may lead to further study of this important question.

The usual method of taking account of soil heterogeneity is the use of check plots. However, in very many cases this method has been far from satisfactory. It is not at all difficult to find examples in the literature of variety tests in which the amount of variation in the check plots is nearly or quite as great as the variation in the other varieties.[1] If check

[1] Davenport, Eugene, and Fraser, W. J. Experiments with wheat, 1888–1895. Experiments with oats, 1888–1895. Ill. Agr. Exp. Sta. Bul. 41, p. 147–160. 1896.
Noll, C. F. Tests of varieties of wheat. Penn. Agr. Exp. Sta. Bul. 115, p. 43–56. 1913.

plots are repeated at sufficiently frequent intervals, they will undoubtedly be a great aid in determining the correction for soil differences. However, where field tests of this kind are carried out on even a moderate scale, the use of check plots adds very materially to the labor and expense of the experiment. For example, in 1914 we grew 150 one-fortieth acre plots. From a study of the field it seems clear that any adequate system of checks would have required 1 check plot to every 5, or about 30 additional plots. The labor involved in handling these would have been considerable; and judging from the literature on the subject, the value of the results might still be very doubtful.

For several years this Station has been carrying on variety tests of oats. The object of these tests is to obtain some measure of the productiveness of new strains or varieties produced in the plant-breeding work. These new strains are always tested along with a number of standard commercial varieties. The method adopted in this work (13) is to grow four systematically repeated plots of each variety. The size of each plot is 33 feet square, or one-fortieth of an acre. The four plots thus make a total of one-tenth of an acre devoted to each variety. These plots have always been grown on a more or less rectangular piece of ground. (See fig. 4.) The fields for these tests have been chosen for their apparent uniformity. However, the resulting yields have always indicated that certain portions of the field were much better or worse in respect to soil fertility than the average of the field as a whole. In certain cases two or more of the four plots of a variety come to lie, say, in certain of these more fertile spots. This tends to produce an unduly high average for that variety.

In order to obtain a correcting value for these different soil conditions, it occurred to us to determine first the probable yield of each plot by the contingency method. This may be done as follows: Take a theoretical field divided into plots as in figure 1. Let a, b, c....l represent the observed yields of the respective plots, of which the mean yield is p. Then, assuming all plots to be planted with the same variety and conditions other than the soil to be uniform, we can obtain the most probable yield of, say, plot a by multiplying the sum ac by the sum aj and dividing by the total al. Proceeding in this way for each plot, we can obtain a calculated yield a', b', c'....l' for each plot. The mean of these calculated yields will be the same as the mean of the observed yield—viz, p.

It is clear that these so-called calculated yields correspond to what Pearson (12) in his work on contingency has designated by r_{uv}, or the value for each square on the hypothesis of independent probability. The difference between the observed and calculated yields would then correspond to what Pearson calls a subcontingency.

The "calculated" yields obtained by this contingency method represent the most probable yields of the respective plots based on the distribution of the observed yields. This method of estimating the probable

yield takes into account the soil differences in both directions across the field. To a certain extent it is dependent upon the assumption that the soil changes in a uniform manner from one side of the field to the other. Harris (6) has pointed out that this is not always the case, but that the diagrams of experimental fields indicate that differences in soil are more likely to occur as a spotting of the field. However, a closer study of the observed yields in many experimental fields indicates that there is a tendency for areas of good soil (high yield) to grade off through areas of medium soil to regions of poor soil. Ordinarily, the changes from one extreme to the other are not abrupt (see fig. 3, 4). The diagrams published by Montgomery (10) indicate this to some extent, although such diagrams do not show the graded changes as well as a study of the actual yields of contiguous plots.

a	b	c
d	e	f
g	h	i
j	k	l

FIG. 1.—Diagram illustrating the method of obtaining the "calculated" yield. (For explanation, see text.)

Further, if the distribution of the high and low "calculated" yields in figures 2 and 3 are compared with the high and low observed yields, it will be seen that the former show approximately the same "spotting" as the latter. This method does tend to lessen the variability and to smooth the results. While it is not ideal and does not obviate all the difficulties, it seems possible that this method may prove useful in estimating soil differences.

For cases like Montgomery's wheat experiment (10) or Mercer and Hall's field trials (9), where there are a number of plots all planted with the same variety, the contingency calculated yields may be used directly. For such experiments these calculated yields represent a smoothing of the original observations. In the case of field trials or variety tests, where different plots have different treatments or are planted with different varieties, such a smoothing tends to mask the actual differences between the plots. In such cases a further procedure is necessary.

In the case of a variety test the yield calculated by this contingency method may be regarded as the most probable yield of any given plot if we suppose the whole field had been planted with a single variety whose average yield was the same as the observed average of all the plots. The deviation of the calculated yield of a given plot from the mean of the field may be taken as a measure of the influence of the soil of that plot as compared with the whole field. Thus, if the calculated yield of a given plot is 10 bushels above the average of the field, it may be taken to mean that the soil on this plot is capable of producing 10 bushels more grain than the soil on the field as a whole.

This figure may be used to correct the observed yield of the corresponding plot. Thus, if the observed yield in a given plot is 80 bushels and the calculated yield is 5 bushels above the average of all the plots, then to make the yield of this plot comparable with the average of the field it would be necessary to reduce the observed yield by 5 bushels. Thus, we may obtain for this plot a "corrected" yield of 75 bushels.

Likewise, where the calculated yield is below the average, it is necessary to add a corresponding amount to the observed yield in order to take account of the deficiency in the soil of that plot.

Expressed in a formula, we may let O equal the observed yield and D the deviation of the calculated yield from the mean of the field. Then the

$$\text{"corrected" yield} = O - D$$

In fields where there are comparatively small differences between the yield of individual plots the direct method of correcting the yield as given above may be used. The corrected yields given in figures 2 and 3 were obtained by this direct method.

In the case of variety tests or experiments where there are likely to be marked differences between individual plots, it will be better to make corrections on a relative rather than an absolute basis. To do this, the deviation of the calculated yield from the mean of the field is determined as before. Next the percentage which each deviation is of the mean is determined. Then this percentage of the observed yield is added to, or subtracted from, the observed yield to obtain the corrected yield. An example will make this clear. Suppose the mean yield of the plots in a field is 70 bushels. The observed yield on a given plot is 80 bushels and the calculated yield of this plot is 77 bushels. Thus, the deviation of the calculated yield from the mean is +7 bushels, which is 10 per cent of the mean (70 bushels). The corrected yield will then be 10 per cent less than the observed; or 10 per cent of 80 equals 8 bushels. The resulting corrected yield will be 72 bushels. By the absolute method the corrected yield would have been 73 bushels. The corrected yields given in figure 4 and Table I have been obtained by this method.

It is next of importance to see whether this "corrected" yield has really obviated any of the difficulties. To test this, use may be made of the criterion of soil homogeneity proposed by Harris (6). This can best be tested upon such data as those furnished by the experimental fields of Montgomery (11) or Mercer and Hall (9).

Figure 2 is a diagram taken from Montgomery (11). It represents a field of Turkey wheat grown in 1908–9. This field was divided into 224 blocks (each 5.5 feet square), as indicated. The grain from each block

671 692	657 697	703 702	755 714	760 703	686 665	592 590	739 712	732 688	720 648	753 646	680 654	680 559	677 684	795 762	723 683
658 672	713 746	613 604	632 583	667 603	645 626	660 652	768 734	786 734	768 698	666 550	843 809	795 767	763 765	716 675	741 693
657 644	671 678	623 587	715 637	543 449	613 557	640 604	798 735	759 678	764 664	995 847	793 731	936 880	755 728	792 722	858 762
642 644	680 701	654 632	673 610	760 682	709 668	682 661	724 677	774 709	860 776	787 657	725 678	664 623	851 838	690 636	770 681
755 744	580 608	620 605	675 620	765 695	742 708	772 758	698 658	652 594	661 584	768 646	777 738	745 712	768 762	852 804	719 665
575 613	598 654	705 720	642 619	704 695	643 640	650 666	572 563	752 726	740 696	863 776	680 672	722 719	723 747	703 688	756 734
727 772	633 696	615 638	685 670	662 632	639 644	657 680	608 607	620 602	624 588	745 666	764 764	703 708	752 784	788 781	682 668
572 664	373 500	560 622	645 682	692 715	644 699	632 705	574 624	606 640	648 666	806 784	791 842	629 684	650 730	679 723	588 626
580 641	425 504	721 771	730 732	706 694	732 734	736 776	655 672	673 673	793 776	765 705	576 593	609 631	568 616	728 738	620 643
588 649	526 605	596 636	777 780	776 765	779 805	721 762	728 745	604 673	742 725	665 606	621 638	611 633	623 671	646 657	617 621
617 682	683 765	726 770	835 842	668 661	664 690	691 735	770 801	775 779	685 672	723 668	583 604	580 606	395 447	511 526	653 661
602 710	662 786	640 720	700 753	650 690	655 727	563 652	600 677	730 781	690 725	713 709	530 597	568 640	410 506	478 539	636 690
665 726	736 815	630 670	598 601	805 883	592 674	593 633	659 686	728 728	705 688	667 608	585 602	560 582	655 704	633 644	733 736
809 682	706 797	790 842	678 753	695 697	715 750	622 675	658 697	597 610	632 628	713 668	585 615	657 692	495 556	618 642	652 668

FIG. 2.—Diagram showing the observed and corrected yield (in grams) of grain on each of Montgomery's wheat plots in 1908–9. The upper figure in each plot is the observed yield and the lower the corrected.

was threshed and weighed separately. The upper figure in each square is the observed yield of grain in grams. The lower figure is the corrected yield obtained by the method outlined above. The mean yield of these plots is taken as 681 gm.

Figure 3 represents the combination plots obtained by grouping the plots in figure 2 in groups of four—i. e., a two- by two- fold grouping. In this figure the upper number in each plot is the observed yield, the lower number the corrected yield, while the middle number is the "calculated" yield. This latter is inserted to illustrate the method of obtaining the corrected yield. The mean yield of these grouped plots is taken as 2,723 gm.

Now, if we calculate the correlation between the observed yield of the ultimate plots and the observed yield of the combination plots it is found that

$$r = +0.358 \pm 0.039$$

This shows a fairly large coefficient of correlation, indicating a relatively large heterogeneity in the soil of this field.

If we calculate the correlation between the corrected yields of the ultimate plots and the corrected yields of the combination plots it is found that

$$r = +0.111 \pm 0.045$$

This coefficient is less than three times its probable error and is hardly to be regarded as significantly greater than 0. In any case it indicates that this method of correcting the yields has practically, if not quite, eliminated the influence of differences in soil of different plots.

2,699 2,616 2,806	2,703 2,826 2,600	2,758 2,894 2,587	2,759 2,798 2,684	2,996 2,953 2,766	2,942 3,007 2,658	2,915 2,766 2,872	2,975 2,887 2,811
2,650 2,707 2,666	2,665 2,924 2,464	2,625 2,994 2,354	2,844 2,895 2,672	3,157 3,056 2,824	3,300 3,112 2,911	3,206 2,862 3,067	3,090 2,987 2,826
2,488 2,594 2,627	2,642 2,802 2,563	2,854 2,869 2,708	2,692 2,774 2,641	2,805 2,928 2,600	3,088 2,982 2,829	2,958 2,743 2,938	3,029 2,863 2,889
2,305 2,414 2,614	2,505 2,608 2,620	2,637 2,670 2,690	2,471 2,583 2,611	2,498 2,725 2,496	3,106 2,776 3,053	2,734 2,553 2,904	2,737 2,664 2,796
2,119 2,444 2,398	2,835 2,640 2,928	2,993 2,703 3,013	2,840 2,614 2,949	2,812 2,759 2,776	2,627 2,809 2,541	2,421 2,584 2,540	2,611 2,697 2,637
2,564 2,345 2,942	2,901 2,533 3,091	2,637 2,593 2,767	2,624 2,508 2,839	2,880 2,647 2,956	2,549 2,694 2,578	1,953 2,479 2,197	2,278 2,587 2,414
2,726 2,421 3,018	2,696 2,615 2,804	2,897 2,677 2,943	2,532 2,589 2,666	2,652 2,732 2,643	2,550 2,782 2,491	2,367 2,559 2,531	2,636 2,671 2,688

FIG. 3.—Diagram showing the observed, corrected, and calculated yield (in grams) of Montgomery's wheat plots in groups of four, taken from figure 2.

Similar coefficients have been calculated for other fields with corresponding results.

It will next be of interest to test this method in the case of an actual variety test. This has been done in the case of all of our own variety test fields. The results will be published in another place in connection with a discussion of some pure-line oat varieties. In order to furnish an example of the use of this method in a variety test, the results of our 1915 test of oat varieties are given below.

Figure 4 represents a diagram of the 1915 plots of oats at the Highmoor Farm (Monmouth, Me.). In the upper left-hand corner of each square is the plot number as it occurs in our records. Immediately below this is the name of the variety. In the case of the pure-line varieties these are

indicated by our own record number—for example, as Maine 340, Maine 357, etc. The upper of the two remaining numbers in each square is the observed yield and the lower number is the corrected yield. All yields are given in bushels per acre.

905 Irish Victor 75.93 78.34	904 Maine 336 73.75 77.11	903 Siberian 72.12 72.02	902 Maine 230 75.25 76.42	901 Banner 73.75 74.85	900 Maine 351 77.75 79.34	899 Swedish Select 68.75 75.16	898 Maine 357 85.94 79.03
913 Maine 247 84.37 85.20	912 Senator 50.83 52.08	911 Maine 281 83.37 81.36	910 Maine 892 82.75 82.19	909 Minn. 26 90.93 90.27	908 Maine 340 82.75 82.60	907 Kherson 53.12 56.99	906 Maine 337 88.75 79.43
921 Maine 286 85.62 85.79	920 Early Pearl 85.00 86.38	919 Maine 346 69.38 67.14	918 Imported Scotch 70.31 69.26	917 Maine 307 76.87 75.70	916 Gold Rain 83.37 82.55	915 Maine 355 73.75 78.57	914 Prosperity 77.50 68.68
929 Maine 336 72.19 72.70	928 Siberian 80.62 82.33	927 Maine 230 74.62 72.61	926 Banner 90.31 89.45	925 Maine 351 78.37 77.59	924 Swedish Select 67.50 67.19	923 Maine 357 73.75 78.93	922 Maine 918 81.25 72.47
937 Senator 61.25 62.35	936 Maine 281 68.75 70.96	935 Maine 978 82.50 81.22	934 Minn. 26 80.25 80.38	933 Maine 340 84.00 84.10	932 Kherson 66.25 66.69	931 Maine 337 81.25 87.79	930 Irish Victor 87.50 79.12
945 Early Pearl 96.50 87.07	944 Maine 346 82.50 75.73	943 Imported Scotch 68.75 59.45	942 Maine 307 82.12 72.59	941 Gold Rain 86.87 76.74	940 Maine 355 93.12 82.83	939 Prosperity 83.37 81.05	938 Maine 247 90.62 70.24
953 Siberian 75.25 74.80	952 Maine 230 76.25 76.90	951 Banner 83.45 80.08	950 Maine 351 73.12 71.45	949 Swedish Select 65.25 63.74	948 Maine 357 82.12 80.67	947 Maine 982 85.62 90.59	946 Maine 286 85.62 75.13
961 Maine 281 81.25 82.94	960 Maine 1053 78.75 81.49	959 Minn. 26 81.25 80.23	958 Maine 340 75.31 75.64	957 Kherson 73.75 74.05	956 Maine 337 73.75 74.45	955 Irish Victor 61.87 67.01	954 Maine 336 84.06 76.27
969 Maine 346 76.87 83.56	968 Imported Scotch 64.65 71.12	967 Maine 307 77.50 81.83	966 Gold Rain 71.56 71.10	965 Maine 355 78.13 83.71	964 Prosperity 70.00 75.36	963 Maine 247 70.00 80.27	962 Senator 60.00 58.88
977 Maine 230 85.62 81.40	976 Banner 85.62 82.68	975 Maine 351 80.62 73.76	974 Swedish Select 71.87 67.08	973 Maine 357 76.87 71.70	972 Maine 1054 84.37 79.19	971 Maine 286 77.81 79.18	970 Early Pearl 90.87 75.35
985 Maine 1064 56.56 60.19	984 Minn. 26 74.37 80.13	983 Maine 340 89.00 91.86	982 Kherson 66.25 69.46	981 Maine 337 71.56 74.99	980 Irish Victor 76.25 80.32	979 Maine 336 65.94 74.10	978 Siberian 83.12 79.43
993 Imported Scotch 55.00 59.38	992 Maine 307 71.25 77.86	991 Gold Rain 77.50 81.23	990 Maine 355 83.75 89.13	989 Prosperity 67.12 71.41	988 Maine 947 75.62 80.83	987 Senator 51.25 58.34	986 Maine 281 91.87 89.39
					996 Maine 286 61.88 62.50	995 Early Pearl 74.06 80.27	994 Maine 346 90.00 81.72
76.74 78.38	76.74 79.45	76.74 75.81	76.74 77.13	76.74 77.09			

FIG. 4.—Diagram showing the yield of oats (in bushels per acre) on the 1915 variety-test field at Highmoor Farm (Monmouth, Me.) Each square represents a one-fortieth acre plot. (For description see text.)

In this field there were tested 11 commercial varieties and 12 pure-line varieties in quadruplicate one-fortieth acre plots. In addition, seven other pure lines were tested in single plots. It will be noted that in the lower row of the figures there are five plots not planted. In order to use this method of correction, it is necessary to assign values to these plots. The best method of doing this is to assign as the observed yield of each such plot the mean yield of the field. This method does not bias the results in either direction.

Table I shows the average yield, both observed and corrected, for the four plots of each commercial variety and for the 12 pure-line varieties. These corrected yields have been obtained by the percentage method described above.

TABLE I.—*Variation constants for the observed and corrected average yields of commercial and pure-line varieties of oats tested in 1915*

COMMERCIAL VARIETIES

Variety.	Observed yield (bushels per acre).	Standard deviation.	Coefficient of variation.	Corrected yield (bushels per acre).	Standard deviation.	Coefficient of variation.
Minnesota No. 26	81.70±2.00	5.94±1.41	7.27±1.74	82.75±1.46	4.34±1.03	5.24±1.25
Early Pearl	86.61±2.80	8.31±1.98	9.59±2.31	82.27±1.61	4.79±1.15	5.82±1.39
Banner	83.28±2.03	6.04±1.44	7.25±1.73	81.77±1.77	5.26±1.25	6.43±1.53
Cold Rain	79.83±1.84	5.48±1.30	6.86±1.64	77.90±1.50	4.48±1.06	5.75±1.37
Siberian	77.78±1.45	4.33±1.03	5.57±1.33	77.14±1.34	4.00± .95	5.18±1.23
Irish Victor	75.39±3.06	9.09±2.16	12.06±2.91	76.19±1.80	5.35±1.27	7.02±1.68
Prosperity	74.50±2.14	6.37±1.51	8.55±2.05	74.13±1.56	4.65±1.10	6.27±1.50
Swedish Select	68.34± .80	2.39± .56	3.50± .83	68.29±1.41	4.20±1.00	6.15±1.47
Kherson	64.68±2.50	7.43±1.77	11.46±2.76	66.79±2.10	6.25±1.49	9.36±2.25
Imported Scotch	64.68± .63	1.88± .44	2.91± .69	64.80±1.83	5.43±1.29	8.38±2.01
Senator	55.84±1.62	4.81±1.14	8.61±2.06	57.92±1.11	3.32± .79	5.73±1.37
Average	73.89	5.64	7.63	73.63	4.73	6.48

PURE-LINE VARIETIES

No. 340	82.77±1.65	4.90±1.16	5.92±1.41	83.55±1.94	5.76±1.37	6.89±1.65
No. 355	82.19±2.44	7.24±1.72	8.81±2.11	83.55±1.26	3.76± .89	4.50±1.07
No. 281	81.31±2.78	8.27±1.97	10.17±2.45	81.16±2.22	6.61±1.57	8.14±1.94
No. 337	78.83±2.27	6.76±1.61	8.58±2.06	79.17±1.79	5.34±1.27	6.74±1.61
No. 247	80.15±2.66	7.92±1.88	9.88±2.16	79.11±1.84	5.47±1.30	6.91±1.65
No. 357	79.67±1.58	4.70±1.12	5.90±1.41	77.58±1.16	3.47± .82	4.47±1.06
No. 230	77.94±1.50	4.47±1.06	5.73±1.37	77.58±1.05	3.12± .74	4.02± .95
No. 346	79.69±2.54	7.56±1.80	9.49±2.28	77.04±2.16	6.41±1.52	8.32±1.99
No. 307	76.94±1.30	3.86± .92	5.02±1.20	77.00±1.13	3.36± .80	4.36±1.04
No. 286	77.73±3.26	9.69±2.31	12.47±3.01	75.65±2.86	8.49±2.02	11.22±2.70
No. 351	77.47± .91	2.73± .65	3.52± .84	75.54±1.04	3.10± .73	4.10± .97
No. 336	73.99±2.19	6.51±1.55	8.79±2.11	75.05± .58	1.74± .41	2.32± .55
Average	79.06	6.22	7.86	78.50	4.72	6.00

From figure 4 it is seen that in many plots the corrected yield varies quite widely from the observed. However, Table I shows that when the four plots of each variety are averaged there are in most cases comparatively slight differences between the two. This point is a strong argument for the efficiency of four systematically repeated plots in reducing the experimental error. There are, however, a few cases in the table

where the corrected average yield is markedly different from the observed. An instance of this is seen in the Early Pearl variety (Table I). The observed average yield of this variety (86.6 bushels) was the highest obtained in 1915. The difference between the yield of this and the Minnesota No. 26 was nearly 5 bushels. The corrected average yield of these two varieties is practically the same, differing only in a fraction of a bushel. By referring back to figure 4 it is found that the high average yield of the Early Pearl was largely due to the influence of two plots, Nos. 945 and 970. These two plots happened to lie in exceptionally good soil. Their observed yields of 96.5 and 90.9 bushels per acre were reduced to the corrected yields of 87 and 75.4 bushels, respectively.

As is to be expected, the corrected average yields show in nearly all cases a much lower variability. This is true of both the absolute and relative variability. In one or two instances, as the Imported Scotch (Table I), the variability is greater in the case of the corrected yield. If all the varieties (Table I) are taken, the corrected yields will show an average decrease in the coefficient of variation of about 1½ per cent.

The table shows that with systematically repeated plots the yields corrected by this method do not differ radically from the actually observed yields. Such changes in the order of yield as do occur we believe more truly express the relative value of these varieties. This statement is based on the experience of several years with these same varieties.

In using this method attention should be called to one or two points. In the first place where a field of plots is very large or where it is relatively long and narrow better results will usually be obtained by breaking it up into smaller blocks for calculation. For example, our 1914 test field was 6 plots wide and 28 plots long. More satisfactory results were obtained by breaking this up into three blocks, two of which were 9 plots long, the other 10 plots. Each block was calculated as a separate field. In doing this, care should be taken that the blocks are not so small as to be unduly affected by a possible preponderance of very good or very poor varieties.

Another point to be remembered in the practical use of this method is that it can not be used to take account of uneven seeding, ravages of birds, or other irregularities in certain plots. Corrections, if any, for these factors should be added before employing the above method.

SUMMARY

It is generally admitted that field trials, including variety tests, are often of very little value because of the large number of uncontrollable factors. Nevertheless, field trials are becoming more and more a necessity in many phases of agricultural investigation.

Within recent years a number of investigators have shown that the experimental error in such trials can be greatly reduced by the use of

systematically repeated plots. Nevertheless, if the number of repetitions is not large, certain experiments may still be unduly influenced by irregularities in the field. It would therefore be desirable if some method could be devised by which the yields of individual plots could be corrected in such a way as to take account of these irregularities.

Check plots have frequently been used for this purpose. But, aside from the extra labor and expense involved, the results from check plots have been far from satisfactory in many cases.

In the present paper a method is proposed for use in correcting for differences in the soil of different plots. The method in its present form is adapted for use only when the plots are arranged in blocks similar to those in figure 4. The method of obtaining this correction factor is as follows: In the first place the probable yield of each plot is obtained by the contingency method. This "calculated" yield represents the most probable yield of each plot on the supposition that they have all been planted with a hypothetical variety whose mean yield is the same as the observed means of the field.

This "calculated" yield may then be used as a basis for determining a correction factor. If the calculated yield of a given plot is above the mean of the field it must be taken that the soil of this plot is better than the average of the field and a corresponding amount must be deducted from the observed yield. Likewise, if the calculated yield is below the average, a proportional amount must be added to the observed yield in order to make the plots comparable.

Still more comparable results will be obtained if the correction factors are based upon the percentage of the mean rather than upon the absolute figures.

Tests of the efficiency of this method by means of the measure of soil heterogeneity proposed by Harris (6) show in all cases a very marked reduction in the amount of heterogeneity when the corrected figures are used. When tested on our own experimental plots, this method leads to results which from other evidence, we have reason to believe, more nearly represent the truth than do the uncorrected yields.

It is realized that this method is not ideal and does not obviate all the difficulties connected with soil differences in plot experiments. It is hoped that this method may prove useful in certain kinds of plot experiments and that it may lead to further study of this problem.

LITERATURE CITED

(1) HALL, A. D.
1909. The experimental error in field trials. *In* Jour. Bd. Agr. [London], v. 16, no. 5, p. 365–370.

(2) ——— and RUSSELL, E. J.
1911. Field trials and their interpretation. *In* Jour. Bd. Agr. [London] Sup. 7, p. 5–14, 2 fig.

(3) HARRIS, J. A.
1912. On the significance of variety tests. *In* Science, n. s. v. 36, no. 923, p. 318–320.

(4) ———
1913. An illustration of the influence of substratum heterogeneity upon experimental results. *In* Science, n. s. v. 38, no. 975, p. 345–346, 1 fig.

(5) ———
1913. Supplementary note on the significance of variety tests. *In* Science, n. s. v. 37, no. 952, p. 493–494.

(6) ———
1915. On a criterion of substratum homogeneity (or heterogeneity) in field experiments. *In* Amer. Nat., v. 49, no. 583, p. 430–454.

(7) HOLTSMARK, G., and LARSEN, B. R.
1906. Über die Fehler, welche bei Feldversuchen durch die Ungleichartigkeit des Bodens bedingt werden. *In* Landw. Vers. Stat., Bd. 65, Heft 1/2, p. 1–22.

(8) LARSEN, B. R.
1898. Om metoder för fältförsök. *In* Ber. Andra Nord. Landtbr. Kong. Stockholm, 1897, bd. 1, Förhandl., p. 72–84. Discussion, p. 85–94.

(9) MERCER, W. B., and HALL, A. D.
1911. The experimental error of field trials. *In* Jour. Agr. Sci., v. 4, no. 2, p. 107–132, 10 fig.

(10) MONTGOMERY, E. G.
1912. Variation in yield and methods of arranging plats to secure comparative results. *In* Nebr. Agr. Exp. Sta. 25th Ann. Rpt. 1910/1911, p. 164–180, 4 fig.

(11) ———
1913. Experiments in wheat breeding: Experimental error in the nursery and variation in nitrogen and yield. U. S. Dept. Agr. Bur. Plant Indus. Bul. 269, 61 p., 22 fig., 4 pl.

(12) PEARSON, KARL.
1904. Mathematical contributions to the theory of evolution. XIII. On the theory of contingency and its relation to association and normal correlation. 35 p., 2 pl. (Drapers' Co. Research Mem. Biomet. Ser. I.)

(13) SURFACE, F. M., and BARBER, C. W.
1914. Studies on oat breeding. I. Variety tests, 1910–1913. Maine Agr. Exp. Sta. Bul. 229, p. 137–192, fig. 53–60.

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